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PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCESMEMORANDUM

Subject: **PARANITROPHENOL. ID# 056301-040510.** Evaluation of Mouse Dermal 18-Month Dermal Carcinogenicity Study, Rat Oral Developmental Toxicity Study and Rat 90-Day Gavage Study.

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**I. CONCLUSIONS**

TB-I has reviewed the 3 submitted studies for paranitrophenol (Guidelines 82-1, 83-2b and 83-3a) and the Executive Summaries are shown below. Data evaluation reports for these 3 studies are attached to this memorandum. In addition, reviews of several mutagenicity studies conducted as part of the NTP mouse dermal carcinogenicity study are attached as additional information on mutagenicity of paranitrophenol. The reviews reflect the conclusions of the HED RfD/Peer Review Committee (meeting of 3/21/96). The rat 90-day gavage study requires submission of information on purity of the test material for upgrading to Acceptable. For the rat developmental toxicity study, TB-I requests provision of information regarding analysis of dosing solutions or, if analysis data are not available, information on the solubility of paranitrophenol in propylene glycol, since no information was provided in the study report.

TB-I will address satisfaction of data requirements for reregistration of paranitrophenol and miscellaneous issues pertaining to other studies in a separate memorandum.

**A. Guideline 82-1a. Rat 90-day gavage study (MRID 42788801).**

**EXECUTIVE SUMMARY:** In a subchronic toxicity study (MRID 42788801), paranitrophenol (tech., % a.i. not provided) was administered for 13 weeks to 20 Sprague-Dawley Crl:CD®BR rats/sex/dose in water by gavage (volume of 10 ml/kg) at dose levels of 0, 25, 70 or 140 mg/kg/day.

At 70 mg/kg/day, 1 male and 1 female died and females showed increased incidence of urine staining between weeks 7 - 9. At 140 mg/kg/day, 14 males and 6 females died and urine staining was observed in females. Clinical signs preceding death included wheezing, dyspnea, pallor, prostration and languid behavior. Death of 1 female at 25 mg/kg/day was not considered treatment-related. There were no compound related effects on body weight, food consumption, hematology, clinical chemistry or organ weight. **The LOEL is 70 mg/kg/day, based on increased incidence of acute mortality and associated clinical signs and pathology, and possibly urine staining in females. The NOEL is 25 mg/kg/day.**

This subchronic toxicity study is classified **Supplementary (upgradable)** and does not satisfy the guideline requirement for a subchronic oral study (82-1a) in rat. However, the study may be upgraded to Acceptable upon submission of the purity of the test material used in this study.

**Note:** This study was submitted instead of a 90-day dermal toxicity study (82-2). Although the 90-day dermal study was required and is the more appropriate study for evaluation of exposure risk from paranitrophenol, a new study is not required at this time. TB-I considers the available data adequate for conducting a risk assessment for dermal exposure to paranitrophenol.

**B. Guideline 83-2b. Mouse 18-month dermal carcinogenicity study (MRID 43766201).**

**EXECUTIVE SUMMARY:** In a dermal carcinogenicity study (MRID 43766201), *p*-nitrophenol (tech., 97.5% a.i.) was administered to 60 Swiss-Webster mice/sex/dose 3 times/week (Monday, Wednesday, Friday) at levels of 0, 40, 80 or 160 mg/kg in 100 µl acetone to the shaved interscapular skin, for 18 months (equivalent to amortized daily doses of 0, 17.1, 34.3 or 68.6 mg/kg/day). Clinical signs, body weight and gross/microscopic pathology were examined.

No treatment-related systemic toxicity was observed. Animals were sacrificed at 18 months due to reduced survival from amyloidosis, observed at high incidence in all

groups. The NOEL for systemic toxicity is  $\geq 160$  mg/kg and the LOEL is  $> 160$  mg/kg.

At the doses tested, there was no treatment-related increase in tumor incidence compared to controls. Dosing was considered adequate based on a mouse 13 week dermal toxicity study in which excessive local dermal irritation at  $\geq 175$  mg/kg and mortality at 350 mg/kg were observed.

This carcinogenicity study in the mouse is **Acceptable** and satisfies the guideline requirement for a carcinogenicity study (83-2b). Study deficiencies (see Discussion of DER) are not considered sufficient to alter the conclusions of the study regarding carcinogenicity of *p*-nitrophenol in mice.

Note: This study was submitted also to satisfy a chronic toxicity study in one species. Although this study does not satisfy this requirement due to lack of evaluation of numerous parameters, no additional data are required. TB-I considers the available data adequate for conducting a risk assessment for dermal exposure to paranitrophenol.

**C. Guideline 83-3a. Rat developmental toxicity study (MRID 42788601).**

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 42788601), paranitrophenol (tech., 99.1% a.i.) was administered to 20 pre-mated female Sprague-Dawley rats/dose in propylene glycol, by gavage at dose levels of 0, 1.4, 13.8 or 27.6 mg/kg/day from days 6 through 16 of gestation. In addition a positive control group (aspirin, 250 mg/kg/day) was included.

At 27.6 mg/kg/day, decreased maternal body weight and weight gain (-12%/-45%) were observed during the dosing period. No treatment-related effects on mortality, clinical signs, food consumption, or cesarean parameters were reported. Food consumption was not measured. **The maternal LOEL is 27.6 mg/kg/day, based on decreased body weight/body weight gain. The maternal NOEL is 13.8 mg/kg/day.**

No treatment-related developmental toxicity was observed. However, the small number of litters (10) available for examination at high dose and lack of some experimental details compromised interpretation of the results. **The developmental NOEL is  $\leq 27.6$  mg/kg/day. A developmental NOEL was not established.**

The developmental toxicity study in the rat is classified as **Supplementary (not upgradable)**, but satisfies the guideline requirement for a developmental toxicity study in rat [OPPTS 870.3700; §83-3(a)]. Although there were insufficient number of litters at high dose, the study is considered acceptable for regulatory purposes and

a new study is not required because no developmental toxicity was observed at doses below the maternal LOEL (see Discussion section for details and study deficiencies).

**D. Mutagenicity Studies Conducted as part of the NTP Mouse Dermal Carcinogenicity Study (contained in MRID 43766201).**

**EXECUTIVE SUMMARY:** In a series of mutagenicity studies conducted as part of the NTP dermal carcinogenicity study in mouse (see MRID 43766201), paranitrophenol was evaluated in the following assays: (1) Salmonella typhimurium reverse gene mutation assay, strains TA98, TA100, TA1535 and TA1537, exposed in the presence or absence of male rat or hamster liver microsomes, negative and positive controls run concurrently; (2) Chinese hamster ovary (CHO) cell cytogenetics assays (sister chromatid exchange or SCE, and chromosomal aberrations, both in presence or absence of male rat liver S9); and (3) Drosophila melanogaster sex-linked recessive lethal mutation assay, using adult Canton-S wild-type flies.

(1) In the Salmonella reverse gene mutation assay, paranitrophenol was not mutagenic (equivocal results in one trial were not reproducible). (2) In the CHO cell cytogenetics assays, paranitrophenol was mutagenic in the chromosomal aberration assay in the presence of S9 but not in the absence of S9 or in the SCE assay. (3) In the Drosophila sex-linked recessive lethal mutation assay, paranitrophenol did not cause increased sex-linked recessive lethal mutations.

(For additional details of these studies, see summary attached to this memorandum; a separate DER was not prepared).

**II. ACTION REQUESTED**

The U.S. Army Aviation and Troop Command, Natick Research, Development and Engineering Center (Natick, MA) submitted a 90-day gavage study in rat, oral (gavage) developmental toxicity study in rat and an 18-month dermal carcinogenicity study in mouse conducted by NTP. These data were submitted by the U.S. Army in support of reregistration of paranitrophenol for use as a fungicide in army boots and in missile silo cork. TB-I notes that the 90-day gavage study was submitted in *lieu* of a 90-day dermal toxicity study and the mouse carcinogenicity study was submitted also to fulfill a chronic dermal toxicity study requirement in one species.

[p-Nitrophenol]

Carcinogenicity Study 83-2(b)

## MUTAGENICITY STUDIES ON P-NITROPHENOL

The following mutagenicity studies were conducted as part of this NTP bioassay on p-nitrophenol (MRID 43766201; mouse cancer study reviewed in this document) and summaries were included in the study report. All were published (see footnotes).

**SALMONELLA TYPHIMURIUM REVERSE GENE MUTATION ASSAY<sup>1</sup>**: Strains TA98, TA100, TA1535 and TA1537 were incubated for 48 hrs at 37°C in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver microsomes. Positive and negative controls were run concurrently. p-nitrophenol was tested in two independent trials at concentrations between 0 and 3333 µg/plate (at least 5 dose levels per trial; high dose reportedly limited by toxicity). Triplicate plates were run for each assay. An assay was considered positive if a reproducible, dose-related increase in revertant colonies was observed in any one strain/activation combination. Assays were considered equivocal when an observed increase in revertants was not dose related, was irreproducible or was of a small magnitude.

Results are summarized in Table C1 taken from the NTP report (attached). p-nitrophenol was not mutagenic under the conditions of this assay. Strain TA98 showed a weakly positive result (2-fold at 333 and 1000 µg/plate) in Trial 1 with hamster S9 but this was not reproducible. Equivocal results were sometimes observed but the magnitude of effects was small and not reproducible in both trials.

**CHINESE HAMSTER OVARY (CHO) CELL CYTOGENETICS ASSAY<sup>2,3</sup>**: Sister chromatid exchange (SCE) was evaluated following treatment of CHO cells for 26 hr in McCoy's 5A medium containing p-nitrophenol in the absence of S9. Bromodeoxyuridine was added 2 hrs after initiation of incubation. Medium containing p-nitrophenol was then replaced with fresh medium containing bromodeoxyuridine and colcemid and the incubation was continued for 2 hrs. In the presence of S9 (from Aroclor 1254-induced male Sprague-Dawley

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<sup>1</sup>Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. and Zeiger, E. (1983) *Salmonella* mutagenicity test results for 250 chemicals. Environ. Mutagen. 5 (Supl. 1), 3-142.

<sup>2</sup>Galloway, S.M. et al. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutagen. 7, 1-51.

<sup>3</sup>Galloway, S.M., et al. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ. Molec. Mutagen. 10 (Supplement 10), 1-175.

[p-Nitrophenol]

Carcinogenicity Study 83-2(b)

rat liver), CHO cells were incubated for 2 hrs with p-nitrophenol, then medium replaced with untreated medium containing bromodeoxyuridine and, for the last 2 - 3 hrs, colcemid. Cells were harvested by mitotic shake-off, fixed and stained with Hoechst 33258 and Giemsa. Fifty second-division metaphase cells with complete karyotype were scored and data were analyzed for trend and pair-wise comparisons. A conservative positive result was identified when SCE frequency was higher than controls by 20%.

Chromosomal aberrations were evaluated following treatment of CHO cells for 19 hrs in McCoy's medium containing p-nitrophenol, followed by a 2-3 hr incubation with colcemid. Cells treated in the presence of S9 were exposed to p-nitrophenol for two hrs, then incubated for 19.5 hr in untreated medium, followed by 2-3 hr with colcemid. Cells were harvested by mitotic shake-off, fixed and stained with Hoechst 33258 and Giemsa. One hundred first-division metaphase cells with complete karyotype were scored for percent of cells with aberrations and data were analyzed for trend and pair-wise comparison. A weak positive response was identified when  $p < 0.05$  for one dose point and a significant trend ( $p < 0.015$ ) were observed. A positive response was identified when significant differences for at least two doses were observed.

The results of the CHO cytogenetics studies are shown in the attached tables taken from the study report. P-nitrophenol did not increase the incidence of SCE under conditions of the assay: a weak positive response in Trial 1 with S9 was not reproducible. P-nitrophenol did not cause increased incidence of chromosomal aberrations in the absence of S9, but a reproducible response (22 to 32% higher incidence than controls) was observed in the presence of S9.

#### DROSOPHILA MELANOGASTERSEX-LINKED RECESSIVE LETHAL MUTATION

**ASSAY<sup>4</sup>:** Adult Canton-S wild-type male flies were exposed to p-nitrophenol either by feeding for 3 days to flies no more than 24 hr-old at 0, 1000, 2500, 6000 or 7500 ppm; administered in 5% sucrose, or by injection into the thorax of 24 to 72 hr-old flies (0, 1000 or 1500 ppm in 0.7% saline and testing at 24 hr post-injection) when feeding did not obtain a response. Dose selection was based on levels that would induce 30% mortality but keep reduced sterility at an acceptable level. Males were mated with females for 3 days, then given fresh females at 2-day intervals for 3 matings of 3, 2 and 2 days, with sample sperm from the successive matings treated at earlier post-meiotic stages. F<sub>1</sub> heterozygous females were mated with their siblings

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<sup>4</sup>Zimmering, S., Mason, J.M., Valencia, R. and Woodruff, R.C. (1985) Chemical mutagenesis testing in *Drosophila*. II. Results of 20 coded compounds tested for the National Toxicology Program. *Environ. Mutagen.* 7:87 - 100.

[p-Nitrophenol]

Carcinogenicity Study 83-2(b)

and placed in individual vials and F<sub>1</sub> daughters of the same male were kept together for identification of clusters (single spontaneous premeiotic mutation events) resulting in several mutant offspring). Vials containing no wild-type males after 17 days identified presumptive lethal mutations and were retested for confirmation. A total of over 5000 treated and 5000 control chromosomes were examined. Recessive lethal data were analyzed using the normal approximation to the binomial test (Margolin et al., 1983). An assay was considered positive when the P value was  $<0.01$  and mutation frequency of the treated group was  $>0.10\%$  or when P was  $<0.05$  and the mutation frequency of the treated group was  $>0.15\%$ . Where P was between 0.01 and 0.05 and the frequency in the treated group was between 0.10% and 0.15%, or P was between 0.05 and 0.10 and the frequency was  $>0.10\%$ , the result was considered inconclusive. When P was  $>0.10$  or frequency was  $<0.10\%$ , the test was considered negative.

Results are summarized in Table C4 (attached) taken from the NTP report. p-nitrophenol did not cause an increase in sex-linked recessive lethal mutations under the conditions of this assay.

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TABLE C1  
Mutagenicity of *p*-Nitrophenol in *Salmonella typhimurium*<sup>a</sup>

Strain	Dose ( $\mu$ g/plate)	Revertants/plate <sup>b</sup>					
		-S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	149 $\pm$ 8.1	195 $\pm$ 5.9	192 $\pm$ 3.9	202 $\pm$ 2.7	182 $\pm$ 7.8	202 $\pm$ 7.8
	10	149 $\pm$ 16.3		184 $\pm$ 4.6		192 $\pm$ 6.1	
	33	140 $\pm$ 4.6		181 $\pm$ 11.3		184 $\pm$ 1.9	
	100	116 $\pm$ 7.1	172 $\pm$ 11.5	185 $\pm$ 3.4	216 $\pm$ 9.9	182 $\pm$ 17.6	221 $\pm$ 11.7
	166		165 $\pm$ 10.1		240 $\pm$ 7.1		224 $\pm$ 18.5
	333	103 $\pm$ 4.5	156 $\pm$ 7.5	198 $\pm$ 6.8	264 $\pm$ 10.1	205 $\pm$ 0.7	249 $\pm$ 7.8
	666		152 $\pm$ 4.0		241 $\pm$ 11.9		194 $\pm$ 11.4
	1,000	104 $\pm$ 7.8	144 $\pm$ 9.1	179 $\pm$ 3.2	142 $\pm$ 11.5	182 $\pm$ 14.3	157 $\pm$ 22.1
	Trial summary	Negative	Negative	Negative	Equivocal	Negative	Equivocal
Positive control <sup>c</sup>		464 $\pm$ 3.2	408 $\pm$ 1.9	847 $\pm$ 55.0	837 $\pm$ 53.6	424 $\pm$ 20.8	510 $\pm$ 25.8
TA1535	0	8 $\pm$ 2.0	10 $\pm$ 1.5	11 $\pm$ 2.5	12 $\pm$ 2.0	10 $\pm$ 1.5	10 $\pm$ 2.1
	10	6 $\pm$ 0.3		16 $\pm$ 1.8		13 $\pm$ 1.2	
	33	11 $\pm$ 1.8		8 $\pm$ 1.5		9 $\pm$ 1.5	
	100	12 $\pm$ 2.3	9 $\pm$ 2.3	14 $\pm$ 2.1	9 $\pm$ 2.1	15 $\pm$ 2.2	13 $\pm$ 1.2
	166		8 $\pm$ 2.0		9 $\pm$ 1.0		8 $\pm$ 1.5
	333	5 $\pm$ 2.0	7 $\pm$ 1.2	9 $\pm$ 1.5	6 $\pm$ 1.2	16 $\pm$ 1.7	10 $\pm$ 3.2
	666		14 $\pm$ 1.2		7 $\pm$ 1.2		8 $\pm$ 0.3
	1,000	7 $\pm$ 2.2	2 $\pm$ 0.3	10 $\pm$ 2.1	5 $\pm$ 0.3	10 $\pm$ 1.9	6 $\pm$ 0.9
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
Positive control		401 $\pm$ 54.9	305 $\pm$ 27.3	103 $\pm$ 9.4	55 $\pm$ 7.2	56 $\pm$ 11.5	76 $\pm$ 2.9
TA1537	0	4 $\pm$ 0.7	3 $\pm$ 1.5	7 $\pm$ 1.2	4 $\pm$ 0.3	8 $\pm$ 2.5	5 $\pm$ 1.5
	10	8 $\pm$ 2.7		12 $\pm$ 0.6		7 $\pm$ 1.0	
	33	5 $\pm$ 1.2		6 $\pm$ 2.3		8 $\pm$ 1.2	
	100	7 $\pm$ 0.7	9 $\pm$ 2.2	10 $\pm$ 1.8	7 $\pm$ 1.9	13 $\pm$ 2.1	6 $\pm$ 0.6
	166		8 $\pm$ 1.2		11 $\pm$ 0.9		9 $\pm$ 2.5
	333	9 $\pm$ 2.6	11 $\pm$ 2.7	22 $\pm$ 5.5	9 $\pm$ 1.5	10 $\pm$ 3.5	9 $\pm$ 1.2
	666		10 $\pm$ 2.1		7 $\pm$ 2.2		8 $\pm$ 1.2
	1,000	11 $\pm$ 1.8	7 $\pm$ 2.0	15 $\pm$ 3.8	<sup>d</sup>	14 $\pm$ 3.2	1 $\pm$ 1.0
	Trial summary	Negative	Negative	Equivocal	Negative	Negative	Negative
Positive control		34 $\pm$ 7.7	38 $\pm$ 9.1	59 $\pm$ 0.7	19 $\pm$ 1.3	33 $\pm$ 9.5	21 $\pm$ 2.1
TA98	0	16 $\pm$ 2.4	17 $\pm$ 4.1	22 $\pm$ 3.8	23 $\pm$ 1.7	26 $\pm$ 2.0	25 $\pm$ 0.9
	10	12 $\pm$ 3.2		26 $\pm$ 2.4		30 $\pm$ 2.2	
	33	14 $\pm$ 1.2		26 $\pm$ 7.0		27 $\pm$ 1.5	
	100	23 $\pm$ 2.5	25 $\pm$ 3.0	20 $\pm$ 2.9	21 $\pm$ 2.4	28 $\pm$ 3.1	21 $\pm$ 1.7
	166		25 $\pm$ 6.0		19 $\pm$ 5.5		23 $\pm$ 2.3
	333	16 $\pm$ 2.3	33 $\pm$ 5.5	41 $\pm$ 9.5	27 $\pm$ 3.8	25 $\pm$ 3.5	24 $\pm$ 1.5
	666		27 $\pm$ 4.7		27 $\pm$ 4.4		21 $\pm$ 5.9
	1,000	15 $\pm$ 1.7	15 $\pm$ 0.3	49 $\pm$ 2.0	19 $\pm$ 2.2	33 $\pm$ 4.7	14 $\pm$ 1.7
	Trial summary	Negative	Equivocal	Weakly Positive	Negative	Negative	Negative
Positive control		226 $\pm$ 7.0	216 $\pm$ 11.9	674 $\pm$ 32.6	346 $\pm$ 38.0	209 $\pm$ 9.8	209 $\pm$ 15.1

<sup>a</sup> Study performed at Case Western Reserve University. The detailed protocol and these data are presented in Haworth *et al.* (1983). The solvent control is 0  $\mu$ g/plate dose.

<sup>b</sup> Revertants are presented as mean  $\pm$  the standard error from three plates.

<sup>c</sup> 2-Aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

<sup>d</sup> Toxic

Table taken from study report NID 43766201

TABLE C2  
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by p-Nitrophenol<sup>a</sup>

Compound	Dose ( $\mu\text{g/mL}$ )	Total Cells	No. of Chromo- somes	No. of SCEs	SCEs/ Chromo- some	SCEs/ Cell	Hrs in BrdU	Relative SCEs/Chromo- some (%) <sup>b</sup>
<b>-S9</b>								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,016	528	0.51	10.6	26.0	
Mitomycin-C	0.001	50	1,016	583	0.57	11.7	26.0	10.42
	0.010	5	106	171	1.61	34.2	26.0	210.42
p-Nitrophenol	0.167	50	1,014	558	0.55	11.2	31.5 <sup>c</sup>	5.89
	0.500	50	1,019	618	0.60	12.4	31.5 <sup>c</sup>	16.70
	1.700	0					31.5 <sup>c</sup>	
								Trend: 2.619
								Probability: 0.004 <sup>d</sup>
Trial 2								
Summary: Negative								
Dimethylsulfoxide		50	1,017	490	0.48	9.8	26.0	
Mitomycin-C	0.001	50	1,024	626	0.61	12.5	26.0	26.88
	0.010	5	104	219	2.10	43.8	26.0	337.06
p-Nitrophenol	5	50	1,017	536	0.52	10.7	33.5 <sup>c</sup>	9.39
	10	50	1,023	529	0.51	10.6	33.5 <sup>c</sup>	7.33
	25	50	1,002	529	0.52	10.6	33.5 <sup>c</sup>	9.58
								Trend: 1.283
								Probability: 0.100
<b>+S9</b>								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		50	1,016	508	0.50	10.2	26.0	
Cyclophosphamide	0.4	50	1,023	774	0.75	15.5	26.0	51.32
	2.0	5	103	250	2.42	50.0	26.0	385.44
p-Nitrophenol	50	50	1,037	500	0.48	10.0	26.0	-3.57
	167	50	1,028	521	0.50	10.4	31.5 <sup>c</sup>	1.36
	500	50	1,030	620	0.60	12.4	31.5 <sup>c</sup>	20.39 <sup>e</sup>
	1,700	0					31.5 <sup>c</sup>	
								Trend: 3.253
								Probability: 0.001

Table taken from study report NTP  
43764201

TABLE C2

Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitrophenol (continued)

Compound	Dose ( $\mu$ g/mL)	Total Cells	No. of Chromo- somes	No. of SCEs	SCEs/ Chromo- some	SCEs/ Cell	Hrs in BrdU	Relative SCEs/Chromo- some (%) <sup>b</sup>
+S9 (continued)								
Trial 2								
Summary: Negative								
Dimethylsulfoxide		50	1,026	475	0.46	9.5	26.0	
Cyclophosphamide	0.4	50	1,032	785	0.76	15.7	26.0	64.30
	2.0	5	104	184	1.76	36.8	26.0	282.16
<i>p</i> -Nitrophenol	1,000	50	1,023	458	0.44	9.2	26.0	-3.30
	1,300	50	1,026	500	0.48	10.0	26.0	5.26
	1,500	50	1,022	518	0.50	10.4	33.5 <sup>c</sup>	9.48
	2,000	0						
								Trend: 1.780
								Probability: 0.038

\* Positive ( $\geq 20\%$  increase over solvent control)

<sup>a</sup> Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1983, 1987).

<sup>b</sup> Percent increase in SCEs/chromosome of culture exposed to *p*-nitrophenol relative to those of culture exposed to solvent.

<sup>c</sup> Because *p*-nitrophenol induced a delay in the cell division cycle, harvest time was extended as needed to maximize the proportion of second division cells available for analysis.

<sup>d</sup> Significance of relative SCEs/chromosome by linear regression trend test vs. log of the dose

Table taken from study report MRO 43766201

TABLE C3

Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by p-Nitrophenol<sup>a</sup>

-S9					+S9				
Dose ( $\mu\text{g/mL}$ )	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose ( $\mu\text{g/mL}$ )	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 - Harvest time: 21.5 hours Summary: Negative					Trial 1 - Harvest time: 21.5 hours Summary: Weak positive				
Dimethylsulfoxide	100	0	0.00	0.0	Dimethylsulfoxide	100	9	0.09	7.0
Mitomycin-C					Cyclophosphamide				
0.040	100	2	0.02	2.0	7.500	100	18	0.18	15.0
0.063	50	5	0.10	10.0	37.500	25	23	0.92	48.0
p-Nitrophenol					p-Nitrophenol				
100	100	5	0.05	4.0	1,500	100	4	0.04	4.0
250	100	0	0.00	0.0	1,750	100	13	0.13	8.0
500	100	1	0.01	1.0	2,000	20	11	0.55	30.0*
1,000	0				2,500	0			
Trend: -0.267 Probability: 0.605 <sup>b</sup>					Trend: 2.172 Probability: 0.015				
Trial 2 - Harvest time: 21.5 hours Summary: Negative					Trial 2 - Harvest time: 21.5 hours Summary: Positive				
Dimethylsulfoxide	100	2	0.02	2.0	Dimethylsulfoxide	100	2	0.02	2.0
Mitomycin-C					Cyclophosphamide				
0.0400	100	12	0.12	12.0	6.250	100	11	0.11	10.0
0.0625	25	8	0.32	28.0	12.500	25	18	0.72	44.0
p-Nitrophenol					p-Nitrophenol				
100	100	0	0.00	0.0	1,250	100	8	0.08	5.0
250	100	0	0.00	0.0	1,500	50	25	0.50	22.0*
500	100	4	0.04	4.0	1,750	50	49	0.98	32.0*
750	0				2,000	0			
Trend: 1.012 Probability: 0.156					Trend: 6.042 Probability: 0.000				

\* Positive ( $P < 0.05$ )<sup>a</sup> Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1983, 1987).<sup>b</sup> Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose

Table taken from study report MRID 43766201

TABLE C4  
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by *p*-Nitrophenol<sup>a</sup>

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			Total <sup>b</sup>
				Mating 1	Mating 2	Mating 3	
Study performed at University of Wisconsin, Madison							
Injection	1,500	37	12	0/2,178	2/1,664	0/1,382	2/5,224 (0.04%)
	0			2/2,499	2/1,882	4/1,460	8/5,841 (0.14%)
Feeding	2,500	12	0	1/2,258	0/1,578	1/1,728	2/5,564 (0.04%)
	0			1/2,202	0/1,678	1/1,817	2/5,697 (0.04%)
Study performed at Brown University							
Feeding	1,000	1	3	0/1,159	0/1,200	1/1,164	1/3,523 (0.03%)
	0			0/1,172	1/1,217	0/1,077	1/3,466 (0.03%)
Injection	1,000	39	16	3/3,304	1/3,467	2/3,278	6/10,049 (0.06%)
	0			7/3,385	4/3,389	1/3,650	12/10,424 (0.12%)
Feeding	6,000	53	0	1/1,744	2/1,767	0/1,748	3/5,259 (0.06%)
	0			1/1,779	0/1,795	2/1,757	3/5,331 (0.06%)
Feeding	7,500	47	0	0/579	1/593	1/495	2/1,667 (0.12%)
	0			0/567	0/593	0/492	0/1,652 (0.00%)

<sup>a</sup> A detailed protocol of the sex-linked recessive lethal assay and the data from the Brown University study are presented in Zimmering *et al.* (1985) and Margolin *et al.* (1983).

<sup>b</sup> Combined total number of lethal mutations/number of X chromosomes tested for three mating trials

Table taken from study report NPL 43766201

[Paranitrophenol]

Subchronic Oral Study [82-1(a)]

EPA Reviewer: Linnea J. Hansen, Ph.D. Linnea Hansen, Date 2/27/96  
Review Section IV, Toxicology Branch I (7509C)  
EPA Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley, Date 3/4/96  
Section Head  
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD
------------------------

STUDY TYPE: Subchronic Oral Toxicity [gavage-rat];  
OPPTS 870.3100 (rodent) [82-1 (a)]

DP BARCODE: D192574

SUBMISSION CODE: S443281

P.C. CODE: 056301

TOX. CHEM. NO.: 603

TEST MATERIAL (PURITY): Paranitrophenol (purity not provided)

SYNONYMS: 4-nitrophenol, PNP, 4-hydroxynitrobenzene,  
parahydroxynitrobenzene

CITATION: Schulze, G.E., Ph.D (1992) Subchronic Toxicity Study  
in Rats with Para-Nitrophenol. Hazleton  
Laboratories America, Inc., Vienna, VA. Laboratory  
study number 241-221, September 21, 1992  
(reformatted report). MRID 42788801. Unpublished  
study.

SPONSOR: U.S. Army Aviation and Troop Command, Natick Research,  
Development and Engineering Center, Natick, MA

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID  
42788801), paranitrophenol (tech., % a.i. not provided) was  
administered for 13 weeks to 20 Sprague-Dawley Crl:CD®BR  
rats/sex/dose in water by gavage (volume of 10 ml/kg) at dose  
levels of 0, 25, 70 or 140 mg/kg/day.

At 70 mg/kg/day, 1 male and 1 female died and females showed  
increased incidence of urine staining between weeks 7 - 9. At  
140 mg/kg/day, 14 males and 6 females died and urine staining was  
observed in females. Clinical signs preceding death included  
wheezing, dyspnea, pallor, prostration and languid behavior.  
Death of 1 female at 25 mg/kg/day was not considered treatment-  
related. There were no compound related effects on body weight,  
food consumption, hematology, clinical chemistry or organ weight.  
**The LOEL is 70 mg/kg/day, based on increased incidence of acute  
mortality and associated clinical signs and pathology, and  
possibly urine staining in females. The NOEL is 25 mg/kg/day.**

This subchronic toxicity study is classified Supplementary  
(upgradable) and does not satisfy the guideline requirement for a  
subchronic oral study (82-1a) in rat. The study may be upgraded  
to Acceptable upon submission of the purity of the test material  
used in this study.

[Paranitrophenol]

Subchronic Oral Study [82-1(a)]

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging Statements were provided. This study was conducted prior to enactment of EPA Good Laboratory Practice Guidelines. The GLP statement stated that "The submitter of the study was neither the sponsor nor conducted it, and does not know whether it had been conducted in accordance with 40 CFR part 160."

## I. MATERIALS AND METHODS

### A. MATERIALS:

#### 1. Test Material: paranitrophenol

Description: granular yellow solid, stored at RT

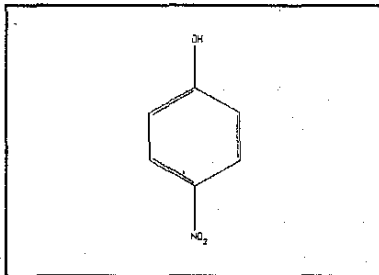
Lot #: AL-DR-080015

Purity: % a.i. not given in study report

Stability of compound: not given in study report; however, reported in other studies to be at least 2 weeks up to 60°C.

CAS #: 100-02-7

[Structure]



#### 2. Vehicle and/or positive control: distilled water.

Lot #: 1028901 (Polar distilled water)

#### 3. Test animals: Species: rat

Strain: Sprague-Dawley Crl:CD®BR

Age and weight at study initiation:

Source: Charles River Laboratories, Raleigh, NC

Housing: individual

Diet: Purina rat chow #5002 ad libitum

Water: Tap water ad libitum

Environmental conditions: Temperature: 64 to 76°C

Humidity: 44 to 70%

Air changes: not stated

Photoperiod: 12 hr light/12 hr dark

Acclimation period: 2 weeks

### B. STUDY DESIGN:

1. In life dates - start: 11-15-88 (start of quarantine)  
end: 3-2-89

[Paranitrophenol]

Subchronic Oral Study [82-1(a)]

2. Animal assignment: Animals were assigned randomly to the test groups in Table 1 using a computerized weight randomization program.

TABLE 1: STUDY DESIGN

Test Group	Dose to Animal (mg/kg/day)	Male	Female
Control	0 (water only)	20	20
Low	25	20	20
Mid	70	20	20
High	140	20	20

3. Dosing solution preparation and analysis

An assumption of 100% purity of the test material a.i. was made for dose solution calculations. Dosing solutions were prepared weekly by mixing appropriate amounts of test substance with a small amount of distilled water to form a paste and mixing with additional water to give the appropriate volume. Solutions were stored at room temperature in amber jars and were sonicated before dosing when crystallization was observed. Prior to study initiation, stability was tested in duplicate samples from dose solutions (control, low and high dose solutions) at Days 0, 7, 10 and 14. During the study, duplicate samples of all dosing solutions prepared for Weeks 1 - 4, 8 and 12 were analyzed for concentration. Homogeneity was not evaluated.

Results - Stability Analysis: Dose solutions remained stable for at least 14 days after preparation. For low and high dose solutions, respectively, the percent of target values were 106 and 108 (Day 0), 102 and 99.3 (Day 7), 99.6 and 102.4 (Day 10) and 98.0 and 95.4 (Day 14).

Concentration Analysis: With the exception of the Week 4 analysis, all dose solutions were within acceptable range of target concentrations (90.7 - 104% of target). The initial week 4 values ranged from 80.0% (70 mg/kg) to 89.3% (140 mg/kg). Following remix and analysis of the formulations 2 days later, values were acceptable. Animals therefore received the low concentrations of test material on only 2 days.

Homogeneity: Although homogeneity was not determined, based on the concentration and stability analyses of

## [Paranitrophenol]

## Subchronic Oral Study [82-1(a)]

duplicate samples, dosing solutions appeared to be homogeneous with the possible exception of the first Week 4 preparations, which were lower than expected (see Concentration Analysis, above).

The analytical data indicated that the mixing procedure was probably adequate and that the variance between nominal and actual dosage to the animals was acceptable. The variations in concentration seen at week 4 in the initial dosing preparations are not considered to significantly affect the study outcome.

4. Administration of test material: Test material was administered by gavage in a dose volume of 10 ml/kg.
5. Statistics - Body weight, body weight change, total food consumption, hematology (excluding cell morphology) and clinical chemistry values, absolute and relative organ weights and organ: brain weight ratios were evaluated statistically. The statistical evaluations used in this study are shown in the attached Figure 1, taken from the study report. Dunnett's t-test was used for group comparisons (two-tailed 5.0% probability level). Cumulative survival was also analyzed using the National Cancer Institute Package. Control vs. treated group comparisons of survival were evaluated by trend analysis of survival at the 5.0%, one-tailed probability level. TB-I has no objection to the methods used.

C. METHODS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality, including a careful examination for clinical signs postdosing. A thorough physical examination was conducted at the weekly weighing.
2. Body weight: Animals were weighed weekly.
3. Food consumption: Food consumption for each animal was determined weekly. Food efficiency values were not calculated.
4. Ophthalmoscopic examination: Eyes were examined by a veterinarian prior to initiation of dosing and prior to scheduled termination of surviving animals.
5. Blood was collected from 10 animals/sex/dose (fasted overnight) at weeks 7 and 14 for hematology and clinical analysis from all surviving animals. The CHECKED (X) parameters were examined.

## [Paranitrophenol]

## Subchronic Oral Study [82-1(a)]

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Corrected leukocyte
X	Leukocyte count (WBC)*		count/cellular morphology
X	Erythrocyte count (RBC)*		Mean corpuscular HGB (MCH)
X	Platelet count*		Mean corpusc. HGB conc. (MCHC)
	Blood clotting measurements*		Mean corpusc. volume (MCV)
	(Thromboplastin time)		Reticulocyte count
	(Thromboplastin time)	X	Methemoglobin (wk 7 only)
	(Clotting time)		
	(Prothrombin time)		

\* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

ELECTROLYTES		OTHER	
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*		Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein (TP)*
			Triglycerides
			Serum protein electrophoresis
ENZYMES			
	Alkaline phosphatase (ALK)		
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase		
	(also SGPT)*		
X	Serum aspartate amino-transferase		
	(also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

\* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis: Not performed in this study (not required for subchronic studies).

7. Sacrifice and Pathology: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination in control and high dose animals. Gross lesions from all groups were examined microscopically. The following tissues were preserved for examination if indicated by signs of toxicity or target organ involvement, but were not examined: eyes, seminal vesicles, mammary gland in females, thigh muscle, prostate, vagina and cervix, femur including articular surface, three levels of spinal cord, exorbital lacrimal glands and skin. The (XX) organs, in

[Paranitrophenol]

Subchronic Oral Study [82-1(a)]

addition, were weighed.

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X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	X	Brain*
X	Salivary glands*	X	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*		Spinal cord (3 levels) <sup>T</sup>
X	Stomach*	X	Lymph nodes*		Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.) <sup>T</sup>
X	Jejunum*	X	Thymus*		
X	Ileum*				
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys**		
X	Rectum*	X	Urinary bladder*		GLANDULAR
XX	Liver**	XX	Testes**	X	Adrenal gland*
	Gall bladder*	XX	Epididymides		Lacrimal gland <sup>T</sup>
X	Pancreas*		Prostate		Mammary gland <sup>T</sup>
			Seminal vesicle	X	Parathyroids***
	RESPIRATORY	X	Ovaries	X	Thyroids***
X	Trachea*	X	Uterus*		
X	Lung*				OTHER
	Nose				Bone
	Pharynx				Skeletal muscle
	Larynx				Skin
					All gross lesions and masses*

\* Required for subchronic studies based on Subdivision F Guidelines

+ Organ weight required in subchronic and chronic studies.

\*\* Organ weight required for non-rodent studies.

T = required only when toxicity or target organ

## II. RESULTS

### A. Observations:

1. Toxicity - No treatment-related clinical signs were observed among surviving animals, with the possible exception of increased incidence of urine staining among mid and high dose females (total of 7 and 10 females affected, respectively) between weeks 7 and 9. The toxicologic significance of this is unclear since it was not observed at later times or in males, and no other signs of toxicity were noted in either sex. Clinical signs observed postdosing in those animals found dead during the study are summarized in the attached Table 2 from the study report and include pallor, languid behavior, wheezing, dyspnea and prostration and are considered to be related to the acute toxic response.
2. Mortality - The attached Table 2, taken from the study report, summarizes the mortality data and includes day of death and associated clinical observations. Significant mortality was observed at the high dose in both sexes but

## [Paranitrophenol]

## Subchronic Oral Study [82-1(a)]

particularly in males: from control to high dose, respectively, 0, 0, 1 and 15 deaths were observed in males and 0, 1, 1 and 6 deaths in females, for a total of 0, 1, 2 and 21 deaths. The study author attributed one death (high dose male) to gavage error. The study author (1) also noted that the deaths of several rats (1 mid-dose male, 2 high-dose males, 3 high dose females) occurred shortly after the bleeding at week 7 and believed that the bleeding may have contributed to their deaths but did not indicate why, and (2) did not consider the death of the low dose female to be treatment-related due to lack of clinical signs. The sudden onset of clinical signs, types of signs and death following dosing in the absence of cumulative toxicity suggest either an acute toxic response or an anaphylactic reaction to the test material.

- B. Body weight and weight gain: No treatment-related effects were observed. Body weight gains at Week 13 (controls to high dose, respectively) in males were 319.2, 327.5, 325.2 and 322.5 g and in females were 168.1, 153.6, 172.3 and 161.3 g.
- C. Food consumption: No treatment-related effects were observed. Food consumption at all intervals was similar among the treatment groups (data now shown).
- D. Ophthalmoscopic examination - No treatment-related observations were observed at the terminal eye examination.
- E. Blood work:
1. Hematology - No treatment-related effects were observed. The following statistically significant changes in hematology parameters in males at Week 14 (see attached Table 5 from study report) were not considered toxicologically significant because they were within normal range: at high dose, increased segmented neutrophils, increased monocytes and reduced eosinophils and at mid dose, increased eosinophils. The study authors noted that at week 7 methemoglobin values for controls were abnormally high, which they attributed to analytical problems. No analyses were done at week 14.
  2. Clinical Chemistry - No treatment-related effects were observed. A statistically significant increase in GGT at low and mid dose in males observed only at week 7 (2 U/ml vs 1 in controls and high dose) was within normal range, did not show a dose response and was not considered treatment-related.

[Paranitrophenol]

Subchronic Oral Study [82-1(a)]

G. Sacrifice and Pathology:

1. Organ weight - No treatment-related effects on absolute organ weights, relative organ weights, or brain to organ weight ratios were observed for the organs that were evaluated.
2. Gross pathology - No treatment-related gross pathology was observed among surviving animals. In the animals that died on study, dark or enlarged liver, lung and kidney were observed (see Table 2, below for summary of incidence).

TABLE 2: SELECTED GROSS OBSERVATIONS IN UNSCHEDULED DEATHS<sup>a</sup>

SEX/OBSERVATION	Dose PNP, mg/kg/day			
	0	25	70	140
Males: N = ( )	(0)	(0)	(1)	(15)
Lung, dark	-	-	0	10
Liver				
thickened	-	-	0	4
dark	-	-	0	7
enlarged	-	-	0	2
Kidney				
dark	-	-	0	2
enlarged	-	-	0	3
Females: N = ( )	(0)	(1)	(1)	(6)
Lung, dark	-	0	1	5
Liver				
thickened	-	0	0	0
dark	-	0	0	2
enlarged	-	0	0	0
Kidney				
dark	-	0	0	0
enlarged	-	0	0	1

a Data extracted from Text Table 5, study report HLA 241-221 (data not analyzed statistically)

- No animals examined (no unscheduled deaths)

3. Microscopic pathology -

- a) Non-neoplastic - Among surviving animals, no treatment-related non-neoplastic lesions were observed. In the animals dying on study, congestion was observed in several organs (incidence summarized below in Table 3) and was probably secondary to respiratory distress and hypoxia preceding sudden death.

[Paranitrophenol]

Subchronic Oral Study [82-1(a)]

TABLE 3: NONNEOPLASTIC MICROSCOPIC LESIONS IN UNSCHEDULED DEATHS<sup>a</sup>

SEX/LESION	Dose PNP, mg/kg/day			
	0	25	70	140
Males: N = ( )	(0)	(0)	(1)	(15)
Pituitary, congestion	-	-	0	9
Adr. cortex, congestion	-	-	0	6
Heart, subacute inflamm.	-	-	0	3
Lung, congestion	-	-	1	14
Liver, congestion	-	-	0	12
Kidney, congestion	-	-	1	13
Females: N = ( )	(0)	(1)	(1)	(6)
Pituitary, congestion	-	0	0	3
Adr. cortex, congestion	-	0	0	5
Heart, subacute inflamm.	-	0	0	0
Lung, congestion	-	0	1	5
Liver, congestion	-	1	1	5
Kidney, congestion	-	1	1	6

a Data extracted from Table 10A of study report # HLA 241-221 (data not analyzed statistically)

- No tissues examined (no unscheduled deaths)

The severity of the congestion observed in these organs was mostly graded as slight or moderate, and occasionally minimal for kidney and liver. Two high dose males showed moderately severe congestion in the liver. The increased incidence of subacute inflammation of the heart in high dose males was not considered a treatment-related effect due to its minimal severity.

b) Neoplastic - No treatment-related neoplastic lesions were observed.

### III. DISCUSSION

A. An increased incidence of urine staining in mid and high dose females was observed, but only between weeks 7 - 9: the toxicologic significance of this finding is unclear but TB-I considers it a possible treatment-related effect. Although there were no overt indications of cumulative toxicity, considerable mortality was observed at high dose in both sexes (none in controls), particularly in males (15 males vs. 6 females; 1 male death due to gavage error). A total of 2 animals died on study at mid dose, and one female at low dose. The rationale for the doses selected was not provided, but the high dose approached the LD<sub>50</sub> for paranitrophenol (191 mg/kg, males and 170 mg/kg, females) as determined in a previously submitted rat acute oral toxicity study (MRID 42539601).

The study authors considered the death of one female at low

## [Paranitrophenol]

## Subchronic Oral Study [82-1(a)]

dose to be unrelated to treatment because of the lack of clinical signs observed and designated a NOEL of 25 mg/kg/day and a LOEL of 70 mg/kg/day. TB-I will accept their conclusions since the significance of a single death at a relatively low dose is unclear. The two animals found dead at mid dose (70 mg/kg/day) showed clinical signs shortly after dosing.

The reason for the acute onset of clinical signs and mortality post-dosing in the absence of observable cumulative toxicity was not clear. One possibility is that paranitrophenol caused an allergic reaction following repeated dosing. The symptoms observed (eg. dyspnea, wheezing) are consistent with an allergic reaction (it is noted that paranitrophenol did not cause dermal sensitization in the study submitted to the Agency, MRID 42540001, but the study was not considered acceptable). Alternatively, deaths may have been due simply to acute toxicity at doses that approached the oral LD<sub>50</sub>, and paranitrophenol given by gavage may not cause overt symptoms at doses approaching the oral LD<sub>50</sub>.

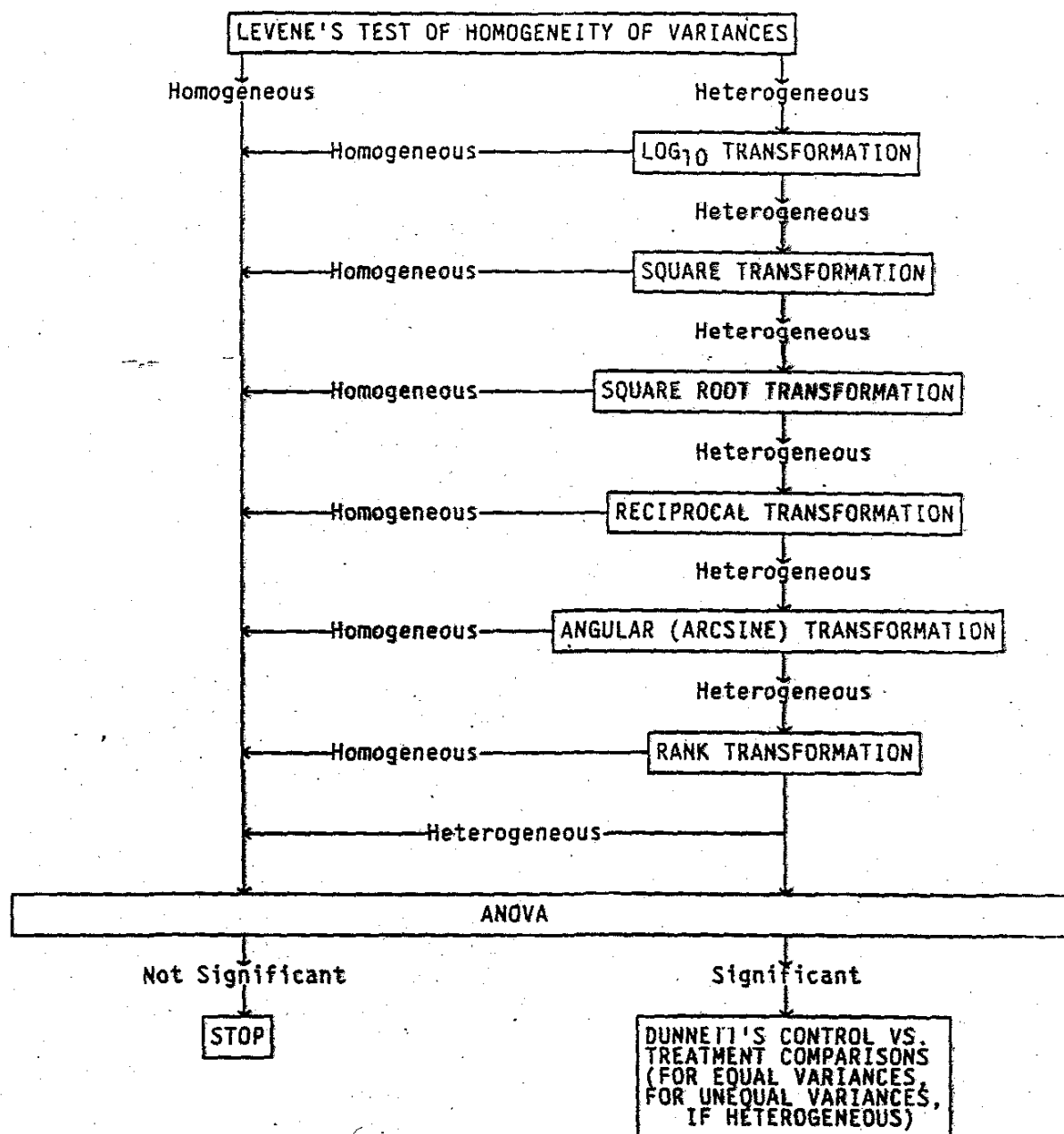
- B. Study deficiencies: The study report did not provide the purity of the test material. This information must be provided for acceptance of the study, although the purity is probably  $\geq 95\%$  based on the analysis of dosing solutions. Significant mortality was observed at the highest dose tested. However, a NOEL may be determined from this study and it may be upgraded to acceptable with submission of test material purity information, which is reportedly on file with the study sponsor.



Figure copied from HLA 241-221

- 20 -

Figure 1  
Statistical Analyses Flowchart



All parametric comparisons take variance homogeneity/heterogeneity into consideration. All transformations indicated in the flowchart are done on untransformed data.



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Table copied from  
Study # HLA 241-221

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Text Table 2

Animal Number	Study Week	Study Day	Death Status	Comments
<b>Males</b>				
<b>Group 3 - 70 mg/kg/day</b>				
890470	7	44	Found dead	Approximately 5 minutes after the animal was noted to be pale, languid, prostrate, with wheezing, and dyspnea.
<b>Group 4 - 140 mg/kg/day</b>				
890502	13	86	Found dead	
890503	7	45	Found dead	
890504	14	92	Found dead	
890505	13	85	Found dead	Approximately 15 minutes after the animal was noted to be languid, prostrate, and with wheezing and dyspnea.
890506	7	49	Found dead	
890507	9	59	Found dead	Noted to have dyspnea the previous day.
890509	14	92	Found dead	
890511	12	79	Found dead	
890513	8	56	Found dead	
890514	8	51	Found dead	Noted to have dyspnea the previous day.
890515	13	85	Found dead	Approximately 15 minutes after the animal was noted to be languid, prostrate, and with wheezing and dyspnea.
890517	7	45	Found dead	Approximately 10 minutes postdose.
890518	8	52	Found dead	Approximately 2 minutes after the animal was noted to be pale, languid, prostrate, and with dyspnea.
890519	12	81	Found dead	
890520	7	43	Found dead	Approximately 5 minutes after the animal was noted to be languid, prostrate, and with dyspnea.
<b>Females</b>				
<b>Group 2 - 25 mg/kg/day</b>				
890459	6	39	Found dead	
<b>Group 3 - 70 mg/kg/day</b>				
890495	7	43	Found dead	Approximately 30 minutes after the animal was noted to be languid, prostrate, and with dyspnea.
<b>Group 4 - 140 mg/kg/day</b>				
890522	7	45	Found dead	Approximately 10 minutes postdose.
890525	7	45	Found dead	Approximately 10 minutes postdose.
890526	7	45	Found dead	Approximately 10 minutes postdose.
890532	10	67	Found dead	
890534	13	85	Found dead	Approximately 15 minutes after the animal was noted to be languid, prostrate and with wheezing, polypnea, and dyspnea.
890541	14	92	Found dead	

HLA 241221

TABLE 5  
SUMMARY OF CLINICAL HEMATOLOGY DATA  
SUBCHRONIC TOXICITY STUDY IN RATS

GROUP	BAND - TH/UL		SEG - TH/UL		LYMPH - TH/UL		MONO - TH/UL		EOSIN - TH/UL		BASO - TH/UL	
	WEEK		WEEK		WEEK		WEEK		WEEK		WEEK	
	7	14	7	14	7	14	7	14	7	14	7	14
MALES												
1 (0 MG/KG)												
MEAN	.0	.0	1.2	1.1	10.4	8.6	.1	.1	.1	.0	.0	.0
S.D.	.00	.00	.58	.74	2.83	2.18	.08	.07	.10	.07	.00	.03
N	10	10	10	10	10	10	10	10	10	10	10	10
2 (25 MG/KG)												
MEAN	.0	.0	.9	.9	9.2	5.5	.1	.1	.1	.1	.0	.0
S.D.	.00	.00	.48	.46	2.47	1.68	.09	.15	.08	.08	.00	.00
N	10	10	10	10	10	10	10	10	10	10	10	10
3 (70 MG/KG)												
MEAN	.0	.0	1.0	.9	9.3	5.3	.1	.2	.2*	.0	.0	.0
S.D.	.03	.00	.39	.46	2.37	1.53	.06	.13	.17	.07	.00	.00
N	10	10	10	10	10	10	10	10	10	10	10	10
4 (140 MG/KG)												
MEAN	.0	.0	1.6	2.1*	8.7	5.2	.1	.1	.1	.1	.0	.0
S.D.	.00	.00	2.18	1.21	3.27	2.37	.09	.08	.08	.05	.00	.00
N	10	5	10	5	10	5	10	5	10	5	10	5
FEMALES												
1 (0 MG/KG)												
MEAN	.0	.0	1.0	.8	8.0	5.2	.1	.1	.0	.1	.0	.0
S.D.	.03	.00	.65	.20	2.75	1.87	.19	.05	.07	.07	.00	.00
N	10	10	10	10	10	10	10	10	10	10	10	10
2 (25 MG/KG)												
MEAN	.0	.0	1.2	.8	8.5	5.8	.0	.1	.1	.0	.0	.0
S.D.	.00	.00	.77	.45	2.08	1.29	.04	.06	.09	.07	.00	.00
N	10	10	10	10	10	10	10	10	10	10	10	10
3 (70 MG/KG)												
MEAN	.0	.0	1.0	.8	7.5	5.1	.1	.1	.1	.1	.0	.0
S.D.	.03	.00	.38	.56	1.78	1.77	.08	.07	.07	.08	.00	.00
N	10	10	10	10	10	10	10	10	10	10	10	10
4 (140 MG/KG)												
MEAN	.0	.0	.8	1.2	8.3	4.9	.0	.2*	.0	.0*	.0	.0
S.D.	.03	.00	.29	.80	2.77	1.82	.04	.13	.05	.04	.00	.00
N	10	10	10	10	10	10	10	10	10	10	10	10

\* Significantly different from control value,  $p \leq 0.05$ .

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Carcinogenicity Study 83-2(b)

EPA Reviewer: Linnea J. Hansen *Linnea Hansen*, Date *2/28/96*  
 Review Section IV, Toxicology Branch I (7509C)  
 EPA Secondary Reviewer: Marion P. Copley *Marion Copley*, Date *3/7/96*  
 Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD
------------------------

STUDY TYPE: Carcinogenicity [dermal]-[mouse]; OPPTS 870.4200  
 [\$83-2 (b)]

DP BARCODE: D219339SUBMISSION CODE: S494072P.C. CODE: 056301TOX. CHEM. NO.: 603TEST MATERIAL (PURITY): 4-Nitrophenol (97.5% a.i.)

SYNONYMS: Paranitrophenol, 4-hydroxynitrobenzene,  
 parahydroxynitrobenzene, PNP

CITATION: Alden, C.J., et al. (1988) Toxicology and Carcinogenesis Studies of p-Nitrophenol (CAS No. 100-02-7) in Swiss-Webster Mice (Dermal Studies). Hazleton Laboratories America, Inc., (Rockville, MD). Laboratory report no. 40510-2; Project No. 12312. March 4, 1988. MRID 43766201. Published (National Toxicology Program Technical Report Series No. 417; NIH Publication No. 93-3148, April, 1993).

SPONSOR: U.S. Army Soldier Systems Command (PROV), Natick  
 Research, Development and Engineering Center, Natick, MA

EXECUTIVE SUMMARY: In a dermal carcinogenicity study (MRID 43766201), p-nitrophenol (tech., 97.5% a.i.) was administered to 60 Swiss-Webster mice/sex/dose 3 times/week (Monday, Wednesday, Friday) at levels of 0, 40, 80 or 160 mg/kg in 100  $\mu$ l acetone to the shaved interscapular skin, for 18 months (equivalent to amortized daily doses of 0, 17.1, 34.3 or 68.6 mg/kg/day). Clinical signs, body weight and gross/microscopic pathology were examined.

No treatment-related systemic toxicity was observed. Animals were sacrificed at 18 months due to reduced survival from amyloidosis, observed at high incidence in all groups. The NOEL for systemic toxicity is  $\geq 160$  mg/kg and the LOEL is  $>160$  mg/kg.

At the doses tested, there was no treatment-related increase in tumor incidence compared to controls. Dosing was considered adequate based on a mouse 13 week dermal toxicity study in which excessive local dermal irritation at  $\geq 175$  mg/kg and mortality at 350 mg/kg were observed.

This carcinogenicity study in the mouse is **Acceptable** and satisfies the guideline requirement for a carcinogenicity study (83-2 (b)). Study deficiencies (see Discussion of DER) are not

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considered sufficient to alter the conclusions of the study regarding carcinogenicity of p-nitrophenol in mice.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance and Data Confidentiality Statements were provided. However, the study was conducted in accordance with FDA GLP regulations, CFR part 58 (study conducted prior to publication of EPA GLP regulations). A flagging statement was not provided.

## I. MATERIALS AND METHODS

### A. MATERIALS:

1. **Test Material:** p-nitrophenol, technical  
 Description: buff to tan flaked solid  
 Lot/Batch #: 730 (E.I duPont de Nemours and Co.,  
 Wilmington, DE)  
 Purity: 97.5% a.i.  
 Stability of compound: stable during study at 4°C  
 (stored refrigerated during study); stable 2  
 weeks up to 60°C  
 CAS #: 100-02-7  
 Structure



2. **Vehicle:** Acetone. Lot/Batch # 2440KVEZ (American Scientific Products)
3. **Test animals:** Species: mouse  
 Strain: Swiss-Webster  
 Age and weight at study initiation: 6 weeks. Weights at study initiation not provided in study report.  
 Source: Charles River Breeding Laboratories, Portage, MI  
 Housing: individual polycarbonate cage, hardwood bedding.  
 Cages rotated every 2 weeks in the racks.  
 Diet: NIH-07 (Ziegler Brothers, Gardners, PA), ad libitum  
 Water: tap water, ad libitum  
 Environmental conditions: Temperature: 20° to 26°C  
 Humidity: 33% to 77%  
 Air changes: 15/hr  
 Photoperiod: 12 hr on/off  
 Acclimation period: 14 days

### B. STUDY DESIGN:

1. **In life dates** - Start: November 7, 1984. End: May 5, 1986

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2. Animal assignment: Animals were assigned randomly to the test groups shown below in Table 1. Test material was applied in 100  $\mu$ l acetone to the interscapular skin region of each animal 3 times/week (Monday, Wednesday, Friday). An interim sacrifice group was not included.

TABLE 1: STUDY DESIGN

Test Group	Conc. per dermal application, mg/kg	Avg. daily dose to animal, mg/kg/day <sup>1</sup>	Main Study 18 months	
			male	female
Control	0	0	60	60
Low (LDT)	40	17.1	60	60
Mid (MDT)	80	34.3	60	60
High (HDT)	160	68.6	60	60

1 Average (amortized) daily dose calculated by averaging for 3 applications/week

3. Dose Selection: Doses were selected based on results of a subchronic dermal toxicity study in Swiss-Webster mice (summary of results included in study report). Doses of 0, 21.9, 43.8, 87.5, 175 or 350 mg/kg p-nitrophenol in acetone were applied to the interscapular skin of 10 mice/sex/dose 3 times/week (Monday, Wednesday, Friday) for 13 weeks. At 350 mg/kg, all males and 8 females died before termination; at 175 mg/kg, 3 males and 1 female died. All deaths except 1 were attributed to treatment. Statistically significantly increased incidences of epidermal inflammation (marked to severe), hyperplasia and hyperkeratosis were observed in both sexes at 175 and 350 mg/kg and necrosis was observed in 2-3 males at these dose levels. The study report did not indicate whether other parameters besides skin lesions were evaluated.
4. Test material preparation and analysis: Dosing solutions were prepared every 2 weeks by mixing appropriate amounts of test substance with acetone vehicle for a constant application volume of 100  $\mu$ l per animal and were stored at -20°C in amber vials. Controls were treated with vehicle only. Stability was tested at study start for dosing solution samples held at room temperature and at 4-month intervals for samples stored frozen, and concentration at 2-month intervals for all dose levels. Homogeneity analyses were not performed.

Results - Stability Analysis: Stable for at least 3 weeks at room temperature and for duration of study at

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-20°C (analytical data not included in study report).

Concentration Analysis: All values were within 10% target concentration (0 to +10%). Separate analyses conducted by a second, referee laboratory for selected samples were similar to those obtained by the study laboratory (within  $\leq 4\%$  of each other).

The analytical data indicated that the variance between nominal and actual dosage to the animals was acceptable. Although homogeneity was not evaluated, based on the consistency of the solutions' concentration measurements, mixing appeared to be adequate.

5. Statistics - Survival probabilities were estimated using the product-limit procedure of Kaplan and Meier (1958). Animals that died from natural causes were included but those found dead from other causes were excluded. The method of Cox (1972) for testing of equality of groups along with Tarone's life table test (1975) were used to identify possible dose-related effects on survival.

Tumor incidences were calculated and statistically evaluated as the ratio of a specific neoplastic lesion to all animals with that organ/tissue examined microscopically, with the following exceptions: the ratio of the lesion to the number of animals on which necropsy was performed was used when (1) tissues were examined grossly for detection of tumors prior to microscopic examination, such as skin, intestine, harderian gland and mammary gland or (2) neoplasms had multiple potential sites of occurrence. Statistical analyses methods included logistic regression analysis, the life table test (Cox, 1972 and Tarone, 1975) for rapidly lethal neoplasms, the Fisher exact test and Cochran-Armitage trend test. The prevalence analysis method of Dinse and Lagakos (1983) was used to adjust for intercurrent mortality. Pair-wise comparisons and dose-related trends were analyzed. Non-neoplastic lesion incidences were calculated and statistically evaluated as the ratio of lesions in all animals with that organ/tissue examined microscopically and were analyzed using logistic regression analysis where prevalence was modeled as a logistic function of treatment and time. The Reviewer has no objections to the analyses used.

#### C. METHODS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality. Clinical signs were recorded weekly for the first 13 weeks, and then at 4-week intervals for the remainder of the study.

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2. Body weight: Animals were weighed weekly for the first 12 weeks of the study and every 4 weeks thereafter.
3. Food consumption: Food consumption was not measured (test material administered dermally). 011875
4. Ophthalmoscopic examination: Not performed (not required for carcinogenicity studies based on Subdivision F Guidelines).
5. Blood was not collected in this study for hematology or clinical analysis (except for leukocyte differential, not required for carcinogenicity studies based on Subdivision F Guidelines).
6. Urinalysis: Urine was not collected in this study (not required for carcinogenicity studies based on Subdivision F Guidelines).
7. Sacrifice and Pathology: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. Organ weights were not measured in this study.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	X	Brain*
X	Salivary glands*	X	Heart* (♀ ONLY)		Periph.nerve*
X	Esophagus*	X	Bone marrow*		Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum* (♀ ONLY)	X	Spleen*		Eyes (optic n.)*
X	Jejunum* (♂ ONLY)	X	Thymus*		
X	Ileum*				
X	Cecum* (♀ ONLY)		UROGENITAL		
X	Colon* (♂ ONLY)	X	Kidneys**		GLANDULAR
X	Rectum* (♂ ONLY)	X	Urinary bladder*	X	Adrenal gland*
X	Liver**	X	Testes**		Lacrimal gland
X	Gall bladder*		Epididymides	X	Mammary gland*
X	Pancreas*	X	Prostate	X	Parathyroid*(♀ ONLY)**
		X	Seminal vesicle	X	Thyroids* (♀ ONLY)**
	RESPIRATORY	X	Ovaries**		
X	Trachea*	X	Uterus*		OTHER
X	Lung*			X	Bone*
X	Nose			X	Skeletal muscle*
	Pharynx				Skin*
	Larynx			X	Mesentery
					All gross lesions and masses*

\* Required for carcinogenicity studies based on Subdivision F Guidelines.

+ Organ weight required in chronic studies.

\*\* Organ weight required for non-rodent studies.

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## II. RESULTS

A. Observations

1. Toxicity - No treatment-related clinical signs were observed. The study report did not provide a summary table or individual animal data for clinical signs. The study report did note that by week 60, some mice from all dose groups including the controls appeared emaciated and edematous and demonstrated clinical signs of anemia and hypoproteinemia, but percent incidence was not provided. Examination of these animals at necropsy reportedly indicated that these signs were secondary to amyloidosis, which was observed in all groups.
2. Mortality - Survival rates at selected times during the study are shown below in Table 2:

TABLE 2: PERCENT SURVIVAL AT SELECTED INTERVALS DURING THE STUDY

	Dermal dose (applied 3X/week)			
	0 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
<b>Males</b>				
Week: 40	97	97	98	97
60	87	87	92	87
68	82	70	73	72
76	52	38	52	42
Term. sac.	48	28*	43	40
<b>Females</b>				
Week: 40	98	97	100	98
60	93	92	97	93
68	78	80	80	77
76	58	48	62	50
Term. sac.	58	43	55	45

1 Data calculated from values in Tables 3 and 4, pp. 24, 25 of study report

\*  $p \leq 0.05$

There did not appear to be A treatment-related effect on survival. Survival began to decrease after about week 60 in all groups. Survival was significantly reduced in the low-dose males at termination, but was not considered treatment-related since a dose-response was not observed. The study authors calculated mean survival days for all deaths in males at 505, 483, 502 and 493 days and in females at 511, 505, 521 and 511 days (control to high dose, respectively). The reduced survival during the last weeks of the study was attributed to amyloidosis, which was observed at high incidence among all groups (see Pathology, below).

- B. Body weight: There were no treatment-related effects on body weight. Mean body weights throughout the study were

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similar among groups. At termination, mean body weights of males (control to high dose) were 36.9, 35.6, 37.0 and 35.9 g and of females were 30.4, 30.4, 29.9 and 29.6 g, respectively.

C. Sacrifice and Pathology:

1. Gross pathology - No treatment-related gross lesions were noted; however, the data were not included in this study report.

2. Microscopic pathology -

a) Non-neoplastic - No treatment-related non-neoplastic lesions were reported in this study. Amyloid deposition was the most frequently observed lesion and was observed at relatively high incidence, often greater than 50%, in many organs or tissues in all groups. Affected organs included adrenal gland, parathyroid and thyroid glands, small intestine, liver, heart, salivary glands, spleen and kidney. Severity of lesions was not indicated. Individual animal data for nonneoplastic lesions were not provided (only data on which organs were examined microscopically), so the total number of animals affected with amyloidosis could not be determined. There was no indication of dermal lesions at the application site: inflammation observed in males at high dose was not considered treatment-related because of the low incidence (4% vs. 0 in all other groups).

b) Neoplastic - The incidence of selected neoplastic lesions is shown in Tables A3 and B3 taken from the study report (see attachments) which summarize statistical analyses of tumor incidence for lesions that showed higher incidence among the treated groups. Historical control data for the Swiss-Webster mouse was not available, according to the study report.

In males, the incidence of lung alveolar/bronchiolar adenoma was significantly increased at 40 and 80 mg/kg and combined adenoma/carcinoma at 40 mg/kg (see table for values). However, incidence among high dose males was comparable to controls and therefore a dose-response was not observed. No other significant increases in tumors were observed, although the overall benign + malignant tumor incidence at 40 and 80 mg/kg (but not 160 mg/kg) was significantly increased. The study report noted that two tumors were observed at the site of treatment in males: a keratoacanthoma at 40 mg/kg and papilloma at 80 mg/kg. These tumors were not considered treatment-related because of the low incidence and because a dose-

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response was not observed.

In females, no statistically significant increases in tumor incidence were reported. The incidence of hemangiosarcoma was increased at 80 and 160 mg/kg but not significantly.

### III. DISCUSSION

- A. No signs of toxicity related to treatment were observed in this study at triweekly doses up to 160 mg/kg. However, it should be noted that a number of parameters usually examined in mouse carcinogenicity studies were not evaluated (see B, below). Dosing in this study was considered adequate despite the lack of treatment-related toxicity because a 13-week range-finding study indicated that dermal irritation and mortality occurred at 175 and 350 mg/kg.

TB-I agreed with the study authors that there were no apparent increases in tumor incidence observed in this study: a significant increase in lung adenoma and combined adenoma/carcinoma in males was observed at low and mid dose but not at high dose. No tumor incidences were significantly increased in females. The conclusion of the Technical Reports Review Subcommittee (a panel of independent scientists reviewing NTP studies) for this study was that there was no evidence of carcinogenic activity of paranitrophenol in male and female Swiss-Webster mice. However, some reviewers questioned the use of the Swiss-Webster strain because of uncertainty as to their sensitivity to paranitrophenol toxicity compared to other strains. This strain was specifically requested by the Sponsor (U.S. Army) but the rationale was not given. It was noted that the usual rodent strains used in NTP studies, B6C3F<sub>1</sub> mice and F344 rats, are generally somewhat resistant to dermal carcinogenesis and therefore would not have been good choices for this study.

- B. Study deficiencies: Several parameters normally evaluated in mouse carcinogenicity studies were not included in this study. No organ weights data were collected, blood analyses were not determined (notably the leukocyte differential count), due to moribundity of animals at the intended time of sampling), and evaluation of some tissue or organs (eg. spinal cord) were lacking. In addition, the study report did not include a summary table or individual animal data for clinical signs, body weight or grossly observed lesions. Individual animal data were provided for microscopic evaluation data but were incomplete: a tissue and organ inventory was provided for each animal but only neoplastic lesions were noted, so that the nonneoplastic lesions in a

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given animal could not be evaluated. No historical control data were reportedly available for comparison with the incidence of lung tumors in males. Homogeneity of the dosing solutions was also apparently not analyzed.

TB-I will accept this study, despite numerous deviations from guideline 83-2b, because the study adequately addresses the question of carcinogenicity of p-nitrophenol following long-term, repeated dermal exposure to mice. Studies from the NTP bioassay series are considered to have been properly conducted for assessment of carcinogenicity and are used by the Agency to satisfy the guideline requirements.

TABLE A3

Statistical Analysis of Primary Neoplasms in Male Mice in the 18-Month Dermal Study of *p*-Nitrophenol

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
<b>Adrenal Gland (Cortex): Adenoma</b>				
Overall rates <sup>a</sup>	4/60 (7%)	7/58 (12%)	6/60 (10%)	6/59 (10%)
Adjusted rates <sup>b</sup>	13.2%	37.3%	20.1%	20.5%
Terminal rates <sup>c</sup>	3/29 (10%)	6/17 (35%)	4/26 (15%)	3/23 (13%)
First incidence (days)	536	503	435	442
Life table tests <sup>d</sup>	P=0.328	P=0.060	P=0.322	P=0.250
Logistic regression tests <sup>d</sup>	P=0.301	P=0.115	P=0.348	P=0.272
Cochran-Armitage test <sup>d</sup>	P=0.393			
Fisher exact test <sup>d</sup>		P=0.245	P=0.372	P=0.361
<b>Liver: Hepatocellular Adenoma</b>				
Overall rates	1/60 (2%)	3/59 (5%)	5/60 (8%)	1/60 (2%)
Adjusted rates	3.4%	14.7%	17.9%	4.2%
Terminal rates	1/29 (3%)	1/17 (6%)	4/26 (15%)	1/24 (4%)
First incidence (days)	552 (T)	545	519	552 (T)
Life table tests	P=0.583N	P=0.167	P=0.082	P=0.720
Logistic regression tests	P=0.569	P=0.192	P=0.092	P=0.720
Cochran-Armitage test	P=0.541N			
Fisher exact test		P=0.303	P=0.103	P=0.752N
<b>Lung: Alveolar/bronchiolar Adenoma</b>				
Overall rates	4/60 (7%)	11/58 (19%)	12/60 (20%)	6/60 (10%)
Adjusted rates	11.2%	35.8%	34.0%	20.2%
Terminal rates	2/29 (7%)	3/17 (18%)	6/26 (23%)	4/24 (17%)
First incidence (days)	409	438	454	442
Life table tests	P=0.409	P=0.014	P=0.024	P=0.282
Logistic regression tests	P=0.467	P=0.034	P=0.028	P=0.341
Cochran-Armitage test	P=0.498			
Fisher exact test		P=0.041	P=0.029	P=0.372
<b>Lung: Alveolar/bronchiolar Carcinoma</b>				
Overall rates	5/60 (8%)	9/58 (16%)	6/60 (10%)	7/60 (12%)
Adjusted rates	16.6%	35.6%	14.6%	27.6%
Terminal rates	4/29 (14%)	4/17 (24%)	1/26 (4%)	6/24 (25%)
First incidence (days)	536	486	293	514
Life table tests	P=0.387	P=0.047	P=0.469	P=0.247
Logistic regression tests	P=0.417	P=0.099	P=0.499	P=0.231
Cochran-Armitage test	P=0.468			
Fisher exact test		P=0.179	P=0.500	P=0.381
<b>Lung: Alveolar/bronchiolar Adenoma or Carcinoma</b>				
Overall rates	9/60 (15%)	20/58 (34%)	17/60 (28%)	11/60 (18%)
Adjusted rates	26.8%	61.4%	42.4%	38.3%
Terminal rates	6/29 (21%)	7/17 (41%)	7/26 (27%)	8/24 (33%)
First incidence (days)	409	438	293	442
Life table tests	P=0.494	P=0.001	P=0.051	P=0.248
Logistic regression tests	P=0.505N	P=0.005	P=0.058	P=0.287
Cochran-Armitage test	P=0.455N			
Fisher exact test		P=0.012	P=0.060	P=0.404

Table taken from study report, MSD 43766201

TABLE A3

Statistical Analysis of Primary Neoplasms in Male Mice in the 18-Month Dermal Study of p-Nitrophenol  
(continued)

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
<b>Tissue NOS: Lipoma</b>				
Overall rates	1/60 (2%)	0/60 (0%)	3/60 (5%)	0/60 (0%)
Adjusted rates	3.4%	0.0%	11.5%	0.0%
Terminal rates	1/29 (3%)	0/17 (0%)	3/26 (12%)	0/24 (0%)
First incidence (days)	552 (T)	- <sup>c</sup>	552 (T)	-
Life table tests	P=0.517N	P=0.607N	P=0.265	P=0.538N
Logistic regression tests	P=0.517N	P=0.607N	P=0.265	P=0.538N
Cochran-Armitage test	P=0.500N			
Fisher exact test		P=0.500N	P=0.309	P=0.500N
<b>All Organs: Hemangiosarcoma</b>				
Overall rates	1/60 (2%)	0/60 (0%)	3/60 (5%)	2/60 (3%)
Adjusted rates	3.4%	0.0%	11.5%	6.6%
Terminal rates	1/29 (3%)	0/17 (0%)	3/26 (12%)	1/24 (4%)
First incidence (days)	552 (T)	-	552 (T)	482
Life table tests	P=0.225	P=0.607N	P=0.265	P=0.433
Logistic regression tests	P=0.206	P=0.607N	P=0.265	P=0.461
Cochran-Armitage test	P=0.242			
Fisher exact test		P=0.500N	P=0.309	P=0.500
<b>All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)</b>				
Overall rates	2/60 (3%)	3/60 (5%)	5/60 (8%)	2/60 (3%)
Adjusted rates	5.3%	10.4%	15.3%	5.1%
Terminal rates	1/29 (3%)	1/17 (6%)	3/26 (12%)	0/24 (0%)
First incidence (days)	423	442	362	437
Life table tests	P=0.543	P=0.389	P=0.200	P=0.651
Logistic regression tests	P=0.579N	P=0.501	P=0.219	P=0.686N
Cochran-Armitage test	P=0.579			
Fisher exact test		P=0.500	P=0.219	P=0.691N
<b>All Organs: Benign Neoplasms</b>				
Overall rates	13/60 (22%)	23/60 (38%)	22/60 (37%)	12/60 (20%)
Adjusted rates	38.4%	76.6%	60.6%	38.3%
Terminal rates	9/29 (31%)	11/17 (65%)	13/26 (50%)	7/24 (29%)
First incidence (days)	409	438	435	442
Life table tests	P=0.386N	P<0.001	P=0.033	P=0.474
Logistic regression tests	P=0.355N	P=0.007	P=0.040	P=0.548
Cochran-Armitage test	P=0.266N			
Fisher exact test		P=0.036	P=0.054	P=0.500N
<b>All Organs: Malignant Neoplasms</b>				
Overall rates	9/60 (15%)	13/60 (22%)	14/60 (23%)	13/60 (22%)
Adjusted rates	28.0%	44.6%	36.9%	41.6%
Terminal rates	7/29 (24%)	5/17 (29%)	6/26 (23%)	8/24 (33%)
First incidence (days)	423	442	293	427
Life table tests	P=0.190	P=0.051	P=0.148	P=0.124
Logistic regression tests	P=0.218	P=0.156	P=0.172	P=0.159
Cochran-Armitage test	P=0.249			
Fisher exact test		P=0.240	P=0.177	P=0.240

Table taken from study report NID 43766201

TABLE A3

Statistical Analysis of Primary Neoplasms in Male Mice in the 18-Month Dermal Study of *p*-Nitrophenol  
(continued)

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
<b>All Organs: Benign and Malignant Neoplasms</b>				
Overall rates	20/60 (33%)	33/60 (55%)	33/60 (55%)	21/60 (35%)
Adjusted rates	57.7%	87.7%	79.1%	58.7%
Terminal rates	15/29 (52%)	13/17 (76%)	18/26 (69%)	11/24 (46%)
First incidence (days)	409	438	293	427
Life table tests	P=0.503	P<0.001	P=0.008	P=0.250
Logistic regression tests	P=0.479N	P=0.002	P=0.009	P=0.350
Cochran-Armitage test	P=0.388N			
Fisher exact test		P=0.013	P=0.013	P=0.500

## (T) Terminal sacrifice

a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

b Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

c Observed incidence at terminal kill

d Beneath the "Vehicle Control" column are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.

e Not applicable; no neoplasms in animal group

Table taken from study report  
MRID 43766201

## Lesions in Female Mice

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TABLE B3

Statistical Analysis of Primary Neoplasms in Female Mice in the 18-Month Dermal Study of *p*-Nitrophenol

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
<b>Lung: Alveolar/bronchiolar Adenoma</b>				
Overall rates <sup>a</sup>	7/60 (12%)	8/60 (13%)	8/60 (13%)	6/60 (10%)
Adjusted rates <sup>b</sup>	16.7%	22.3%	19.9%	18.0%
Terminal rates <sup>c</sup>	4/35 (11%)	2/26 (8%)	5/33 (15%)	3/27 (11%)
First incidence (days)	438	449	446	454
Life table tests <sup>d</sup>	P=0.476N	P=0.360	P=0.479	P=0.609N
Logistic regression tests <sup>d</sup>	P=0.403N	P=0.491	P=0.500	P=0.501N
Cochran-Armitage test <sup>d</sup>	P=0.407N			
Fisher exact test <sup>e</sup>		P=0.500	P=0.500	P=0.500N
<b>Lung: Alveolar/bronchiolar Carcinoma</b>				
Overall rates	4/60 (7%)	0/60 (0%)	4/60 (7%)	5/60 (8%)
Adjusted rates	11.4%	0.0%	9.0%	18.5%
Terminal rates	4/35 (11%)	0/26 (0%)	1/33 (3%)	5/27 (19%)
First incidence (days)	552 (T)	- <sup>e</sup>	427	552 (T)
Life table tests	P=0.154	P=0.106N	P=0.631	P=0.338
Logistic regression tests	P=0.205	P=0.106N	P=0.632N	P=0.338
Cochran-Armitage test	P=0.206			
Fisher exact test		P=0.059N	P=0.641N	P=0.500
<b>Lung: Alveolar/bronchiolar Adenoma or Carcinoma</b>				
Overall rates	11/60 (18%)	9/60 (15%)	12/60 (20%)	11/60 (18%)
Adjusted rates	27.4%	24.1%	28.8%	35.1%
Terminal rates	8/35 (23%)	2/26 (8%)	7/33 (21%)	8/27 (30%)
First incidence (days)	438	449	427	454
Life table tests	P=0.366	P=0.589N	P=0.473	P=0.395
Logistic regression tests	P=0.475	P=0.426N	P=0.517	P=0.576
Cochran-Armitage test	P=0.466			
Fisher exact test		P=0.404N	P=0.500	P=0.593N
<b>Lung: Alveolar/bronchiolar Carcinoma or Adenosquamous Carcinoma</b>				
Overall rates	4/60 (7%)	1/60 (2%)	5/60 (8%)	5/60 (8%)
Adjusted rates	11.4%	2.4%	11.9%	18.5%
Terminal rates	4/35 (11%)	0/26 (0%)	2/33 (6%)	5/27 (19%)
First incidence (days)	552 (T)	497	427	552 (T)
Life table tests	P=0.191	P=0.260N	P=0.486	P=0.338
Logistic regression tests	P=0.250	P=0.211N	P=0.516	P=0.338
Cochran-Armitage test	P=0.249			
Fisher exact test		P=0.182N	P=0.500	P=0.500
<b>Uterus: Stromal Polyp</b>				
Overall rates	4/60 (7%)	2/60 (3%)	6/60 (10%)	1/60 (2%)
Adjusted rates	10.4%	6.5%	14.3%	3.7%
Terminal rates	3/35 (9%)	1/26 (4%)	2/33 (6%)	1/27 (4%)
First incidence (days)	463	500	401	552 (T)
Life table tests	P=0.277N	P=0.448N	P=0.368	P=0.245N
Logistic regression tests	P=0.231N	P=0.359N	P=0.375	P=0.186N
Cochran-Armitage test	P=0.235N			
Fisher exact test		P=0.340N	P=0.372	P=0.182N

Table taken from study report MWD 43746201

TABLE B3

## Statistical Analysis of Primary Neoplasms in Female Mice in the 18-Month Dermal Study of p-Nitrophenol (continued)

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
<b>Uterus: Stromal Sarcoma</b>				
Overall rates	1/60 (2%)	2/60 (3%)	1/60 (2%)	3/60 (5%)
Adjusted rates	2.0%	5.9%	3.0%	9.1%
Terminal rates	0/35 (0%)	1/26 (4%)	1/33 (3%)	1/27 (4%)
First incidence (days)	475	482	552 (T)	517
Life table tests	P=0.218	P=0.461	P=0.754	P=0.283
Logistic regression tests	P=0.240	P=0.502	P=0.762	P=0.305
Cochran-Armitage test	P=0.238			
Fisher exact test		P=0.500	P=0.752N	P=0.309
<b>Uterus: Stromal Polyp or Stromal Sarcoma</b>				
Overall rates	5/60 (8%)	4/60 (7%)	7/60 (12%)	4/60 (7%)
Adjusted rates	12.2%	12.2%	17.1%	12.6%
Terminal rates	3/35 (9%)	2/26 (8%)	3/33 (9%)	2/27 (7%)
First incidence (days)	463	482	401	517
Life table tests	P=0.556N	P=0.615N	P=0.375	P=0.586N
Logistic regression tests	P=0.495N	P=0.513N	P=0.383	P=0.505N
Cochran-Armitage test	P=0.500N			
Fisher exact test		P=0.500N	P=0.381	P=0.500N
<b>All Organs: Hemangiosarcoma</b>				
Overall rates	0/60 (0%)	1/60 (2%)	3/60 (5%)	3/60 (5%)
Adjusted rates	0.0%	1.9%	8.5%	11.1%
Terminal rates	0/35 (0%)	0/26 (0%)	1/33 (3%)	3/27 (11%)
First incidence (days)	-	450	545	552 (T)
Life table tests	P=0.052	P=0.500	P=0.121	P=0.079
Logistic regression tests	P=0.067	P=0.494	P=0.126	P=0.079
Cochran-Armitage test	P=0.068			
Fisher exact test		P=0.500	P=0.122	P=0.122
<b>All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)</b>				
Overall rates	4/60 (7%)	2/60 (3%)	5/60 (8%)	2/60 (3%)
Adjusted rates	9.4%	4.0%	13.8%	4.0%
Terminal rates	2/35 (6%)	0/26 (0%)	3/33 (9%)	0/27 (0%)
First incidence (days)	373	449	507	463
Life table tests	P=0.408N	P=0.391N	P=0.487	P=0.379N
Logistic regression tests	P=0.371N	P=0.333N	P=0.505	P=0.338N
Cochran-Armitage test	P=0.368N			
Fisher exact test		P=0.340N	P=0.500	P=0.340N
<b>All Organs: Benign Neoplasms</b>				
Overall rates	15/60 (25%)	10/60 (17%)	15/60 (25%)	14/60 (23%)
Adjusted rates	34.1%	28.8%	34.8%	37.5%
Terminal rates	9/35 (26%)	4/26 (15%)	8/33 (24%)	7/27 (26%)
First incidence (days)	370	449	401	411
Life table tests	P=0.380	P=0.372N	P=0.554	P=0.489
Logistic regression tests	P=0.484	P=0.194N	P=0.579	P=0.500N
Cochran-Armitage test	P=0.479			
Fisher exact test		P=0.184N	P=0.583N	P=0.500N

table taken from study report NAD 43766201

**TABLE B3**  
**Statistical Analysis of Primary Neoplasms in Female Mice in the 18-Month Dermal Study**  
**of *p*-Nitrophenol (continued)**

	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
<b>All Organs: Malignant Neoplasms</b>				
Overall rates	13/60 (22%)	8/60 (13%)	16/60 (27%)	17/60 (28%)
Adjusted rates	30.9%	18.2%	36.0%	46.6%
Terminal rates	8/35 (23%)	1/26 (4%)	7/33 (21%)	10/27 (37%)
First incidence (days)	373	375	427	463
Life table tests	P=0.070	P=0.300N	P=0.331	P=0.140
Logistic regression tests	P=0.097	P=0.166N	P=0.349	P=0.259
Cochran-Armitage test	P=0.096			
Fisher exact test		P=0.168N	P=0.335	P=0.264
<b>All Organs: Benign and Malignant Neoplasms</b>				
Overall rates	25/60 (42%)	16/60 (27%)	28/60 (47%)	26/60 (43%)
Adjusted rates	52.0%	39.9%	56.8%	63.9%
Terminal rates	14/35 (40%)	5/26 (19%)	13/33 (39%)	14/27 (52%)
First incidence (days)	120	375	401	411
Life table tests	P=0.152	P=0.228N	P=0.356	P=0.273
Logistic regression tests	P=0.218	P=0.058N	P=0.317	P=0.500
Cochran-Armitage test	P=0.217			
Fisher exact test		P=0.062N	P=0.357	P=0.500

**(T) Terminal sacrifice**

<sup>a</sup> Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.

<sup>b</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> Beneath the "Vehicle Control" column are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

<sup>e</sup> For all tests, a negative trend or a lower incidence in a dose group is indicated by N.

<sup>e</sup> Not applicable; no neoplasms in animal group

*Table taken from study report MRID 43766201*

Paranitrophenol

Developmental Study OPPTS 870.3700 [§83-3(a)]

EPA Reviewer: Linnea J. Hansen, Ph.D. *Linnea J. Hansen* Date *3/23/96*  
Review Section IV, Toxicology Branch I (7509C)  
EPA Secondary Reviewer: Marion Copley, D.V.M., D.A.B.T., Sec.  
Head *Marion Copley*, Date *3/23/96*  
Review Section IV, Toxicology Branch I (7509C)

## DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - rat; OPPTS 870.3700  
[§83-3 (a)]

DP BARCODE: D192573

SUBMISSION CODE: S443280

P.C. CODE: 056301

TOX. CHEM. NO.: 603

TEST MATERIAL (PURITY): Paranitrophenol (99.1% a.i.)

SYNONYMS: 4-nitrophenol, PNP, 4-hydroxynitrobenzene,  
parahydroxynitrobenzene

CITATION: Angerhofer, R.A. and Weeks, M.H. (1992) Effect of  
Paranitrophenol on the Embryonic Development of  
Rats. U.S. Army Environmental Hygiene Agency, HSHB-  
MO-T, Aberdeen Proving Ground, MD. Laboratory study  
number 75-51-0047-79-F, September 16, 1992 (reformat  
of study originally submitted August 21, 1979).  
MRID 42788601. Unpublished study.

SPONSOR: U.S. Army Aviation and Troop Command, Natick Research,  
Development and Engineering Center, Natick, MA

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID  
42788601), paranitrophenol (tech., 99.1% a.i.) was administered  
to 20 pre-mated female Sprague-Dawley rats/dose in propylene  
glycol, by gavage at dose levels of 0, 1.4, 13.8 or 27.6  
mg/kg/day from days 6 through 16 of gestation. In addition a  
positive control group (aspirin, 250 mg/kg/day) was included.

At 27.6 mg/kg/day, decreased maternal body weight and weight gain  
(-12%/-45%) were observed during the dosing period. No  
treatment-related effects on mortality, clinical signs, food  
consumption, or cesarean parameters were reported. Food  
consumption was not measured. The maternal LOEL is 27.6  
mg/kg/day, based on decreased body weight/body weight gain. The  
maternal NOEL is 13.8 mg/kg/day.

No treatment-related developmental toxicity was observed. How-  
ever, the small number of litters (10) available for examination  
at high dose and lack of some experimental details compromised  
interpretation of the results. The developmental NOEL is  $\leq 27.6$   
mg/kg/day. A developmental NOEL was not established.

The developmental toxicity study in the rat is classified as

## Paranitrophenol

Developmental Study OPPTS 870.3700 [§83-3(a)]

Supplementary (not upgradable), but satisfies the guideline requirement for a developmental toxicity study in rat [OPPTS 870.3700; §83-3(a)]. Although there were insufficient number of litters at high dose, the study is considered acceptable for regulatory purposes and a new study is not required because no developmental toxicity was observed at doses below the maternal LOEL (see Discussion section for details and study deficiencies).

**COMPLIANCE:** Signed and dated GLP, Quality Assurance and Data Confidentiality statements were not provided. The study was conducted prior to publication of the EPA GLP standards of 1988. The study was conducted according to FDA guidelines for reproductive studies for safety evaluation of drugs for human use, 1966. A flagging statement was provided (study did not meet or exceed applicable criteria).

**I. MATERIALS AND METHODS****A. MATERIALS****1. Test Material:** Paranitrophenol, tech.

Description: buff-colored flaked solid. Stability information not provided in this report (reported in other studies to be stable indefinitely at 4°C and for at least 2 weeks stored up to 60°C).

Lot/Batch #: 777A (E.I DuPont deNemours)

Purity: 99.1% a.i.

CAS #: 100-02-7

[Structure]

**2. Vehicle:** Propylene glycol

Description: Not provided

Lot/Batch #: 04102875 (Mallinckrodt)

Purity: not indicated

**3. Test animals:** Species: rat

Strain: Sprague-Dawley

Age at mating: not indicated (females were received pre-impregnated)

Weight at mating: not indicated: pretest weight was between 168 - 230 g

Source: Maryland Breeding Farms, Hewitt, NJ

Housing: two/cage

## Paranitrophenol

Developmental Study OPPTS 870.3700 [§83-3(a)]

Diet: Purina Lab Blox, ad libitumWater: tap, ad libitum

Environmental conditions:

Temperature: 24±1°F

Humidity: 40-50%

Air changes: not indicated

Photoperiod: 12 hrs dark/12 hrs light

Acclimation period (P): none (premated females received and assigned immediately to study groups)

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: June 14, 1978; end: June 30, 1978
2. Mating: The study report stated that premated females were received from the supplier and did not provide details of the mating procedures used by the supplier. The report stated that females were received between days 2 - 4 of gestation.
3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was random.

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0.0	20
Low (LDT)	1.4	20
Mid (MDT)	13.8	20
High (HDT)	27.6	20
Aspirin (Pos. Control)	250.0	20

4. Dose selection rationale: High dose is 1/5 of the oral LD<sub>50</sub>.
5. Dosage preparation and analysis: Dosing solutions were prepared as 0.7, 6.9 and 13.8 mg/ml solutions in propylene glycol for administration at 2 ml/kg. The study report did not provide any information regarding preparation or storage of the dosing solutions, frequency of dose preparation or analysis of dosing solutions for stability, concentration or homogeneity.
6. Dosage administration: All doses were administered once daily by gavage on gestation days 6 through 16 in a volume of 2 ml/kg body weight per day. Dosing was based on that day's body weight.

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**C. OBSERVATIONS**

1. Maternal Observations and Evaluations - The study report did not indicate whether daily observations for clinical signs were conducted. Body weight data were recorded pretest (day of gestation on which pretest measurements were recorded was not indicated), daily on gestation days 6 - 16 and on Day 21. Food consumption was not evaluated. Dams were sacrificed on day 21 of gestation by intracardiac injection of Barb-Euthol®. Examinations at sacrifice consisted of number, location, viability of conceptuses, number of resorptions, number of corpora lutea. No further details were provided.
2. Fetal Evaluations - The fetuses were examined in the following manner: all fetuses were weighed, the length measured and examined visually for external defects. One-third of the fetuses were fixed in Bouin's fluid and examined by the Wilson technique for neural and visceral defects. The remaining two-thirds of the fetuses were fixed in 95% ethanol, cleared and stained with Alizarin Red for examination of skeletal abnormalities (method unspecified).

**D. DATA ANALYSIS**

1. Statistical analyses: The study report did not indicate which methods were used for statistical analysis. Cesarean data were analyzed statistically except for parameters calculated by the reviewer (see Table 3 of DER). Body weight and fetal observation data were apparently not analyzed statistically.
2. Indices: The indices shown in the attached Table II taken from the study report were calculated from cesarean section records of animals in the study. However, it is noted that some of these, eg late vs. early resorption, were not given in the study and that calculation methods for some indices in the cesarean data table were not provided. Several parameters were calculated by the reviewer (see Table 3 of DER).
3. Historical control data: Historical control data were not provided.

**II. RESULTS****A. MATERNAL TOXICITY**

1. Mortality and Clinical Observations: The study authors did not report treatment-related mortality or clinical findings, but did not indicate whether dams were given

## Paranitrophenol

Developmental Study OPPTS 870.3700 [§83-3(a)]

careful daily examinations. One high dose female died (day of death unspecified) due to apparent gavage error and was excluded from the study. No summary or individual animal tables were provided for clinical findings.

2. Body Weight - Body weight gain data are summarized in Table 2, below:

TABLE 2 Maternal Body Weight Gain and Day 21 Body Weights (g)<sup>a</sup>

Interval	Dose in mg/kg/day (# of Dams)				
	Control (15)	Aspirin (17)	LDT (15)	MDT (16)	HDT (10)
Pretreatment: Days ? - 6	23	22.4	22.1	23.8	22.2
Treatment: Days 6 - 16	50.4	24.1	43.2	43.9	27.3
Posttreatment: Days 16 - 21	46	24.4	50.5	47.3	46.5
Mean body wt.					
Pretest	185.5	191.4	186.2	187.1	177.4
Day 16	258.9	237.9	251.5	254.8	226.9
Day 21	304.9	262.3	302.0	302.1	273.4

- <sup>a</sup> Data extracted from (study or report number and tables or appendices used). Means calculated by reviewer (no body weight summary table in study report). Data not analyzed statistically.

The mean body weight and body weight gain of high dose females were decreased relative to controls during the treatment period (-12%/-45%). Body weight gain during the posttreatment period was similar to controls. Females treated with aspirin showed similar decreases, but gain during the post-treatment period was also reduced. TB-I agreed with the study authors that slight decreases in gain during treatment in the low and mid dose females were not biologically significant. Since no food consumption data were available, it could not be determined whether decreases were associated with decreased food consumption. The study report did not provide gravid uterine or adjusted maternal body weight data.

The study report also provided individual animal weights for the surviving non-pregnant females, which were maintained on treatment during the study. There were no apparent treatment-related effects on body weight among these animals (data not shown).

3. Food Consumption - No data were provided.
4. Gross Pathology - The study report stated that there were no signs of gross pathology at necropsy (no data were provided).
5. Cesarean Section Data - Data are as follows as summarized in Table 3, below:

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TABLE 3 Cesarean Section Observations<sup>a</sup>

Observation	Dose (mg/kg/day)				
	0	ASPIRIN/250	PNP/1.4	PNP/13.8	PNP/27.6
# Animals Assigned (Mated)	20	20	20	20	20
# Animals Pregnant Pregnancy Rate (%)	15 (75)	17 (85)	15 (75)	16 (80)	11 (55)
# Nonpregnant	5	3	5	4	9
Maternal Wastage					
# Died	0	0	0	0	1
# Died Pregnant	0	0	0	0	0
# Died Nonpregnant	0	0	0	0	1
# Aborted	0	0	0	0	0
# Premature Delivery	0	0	0	0	0
Total # Corpora Lutea <sup>b</sup>	185	188	177	206	130 <sup>c</sup>
Corpora Lutea/Dam <sup>b</sup>	12.33	11.06	11.80	12.88	13.00 <sup>c</sup>
Total # Implantations	166	167	151	177	106 <sup>c</sup>
Implantations/Dam	11.07	9.82	10.07	11.06	10.2 <sup>c</sup>
Total # Litters	15	12	15	16	10 <sup>c</sup>
Total # Live Fetuses	154	96	145	164	102 <sup>c</sup>
Live Fetuses/Dam	10.27	5.65*	9.73	10.25	10.2 <sup>c</sup>
Total # Dead Fetuses	1	0	0	0	0
Dead Fetuses/Dam	0.067	0	0	0	0
Total # Resorptions	11	71	5	13	4 <sup>c</sup>
Early	-	-	-	-	-
Late	-	-	-	-	-
Resorptions/Dam	0.73	4.18*	0.33	0.81	0.40 <sup>c</sup>
Early	-	-	-	-	-
Late	-	-	-	-	-
Litters w/Total Resorptions	0	5	0	0	0
Mean Fetal Weight (g)	3.91	2.53	4.3	4.22	4.27
Males	-	-	-	-	-
Females	-	-	-	-	-
Sex Ratio (% Male) <sup>b</sup>	44	54	46	54	49
Preimplantation Loss (%) <sup>b</sup>	10.27	11.17	14.69	14.08	18.46 <sup>c</sup>
Postimplantation Loss (%) <sup>b</sup>	7.23	45.51	3.97	7.34	3.77 <sup>c</sup>

a Data extracted from Table III and Appendix C, study report #75-51-0047-79-F

b Calculations excluded one pregnant female (#713) sacrificed on Day 19 by mistake because the individual data for this animal was not included in the study report. See text for discussion.

c Calculated by reviewer (means not calculated by study author)

\*  $p \leq 0.05$

- Data not available or means not calculated from individual animal data

No treatment-related effects were observed on cesarean parameters. Fertility was low, especially in the high dose group. The study

## Paranitrophenol

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authors speculated that low fertility may have been because the females were immature, based on body weights being lower than expected at receipt. In the high dose group there were only 11 pregnant females and 10 litters for examination at day 21 due to accidental sacrifice of one pregnant female (#713) on Day 19. The study report stated that this female was examined and fetuses were reportedly small and of normal gross morphology, but the individual animal data for #713 was not included in the study report and no fetuses from this litter were examined for visceral or skeletal abnormalities. In Table III from the study report, some high dose group values differed from those obtained by the reviewer by addition of individual animal data: implantation (eg total implantations 115 vs. 106), live fetus (total 107 vs. 102) and resorptions (8 vs. 4). It appears that the study report included data from female #713 but this could not be confirmed due to lack of individual data for this female. However, the conclusions are the same using either numbers.

Increased preimplantation loss was observed at the high dose but was not considered treatment-related since this would be expected to occur prior to treatment. Preimplantation loss may have been related to transport of the animals following fertilization and lack of an acclimatization period. The aspirin positive control showed pronounced effects on resorptions, post-implantation loss, fetal viability and fetal weight.

**B. DEVELOPMENTAL TOXICITY:** Selected skeletal findings are shown below in Table 4.

1. External Examination - No external abnormalities were observed among the vehicle control or treatment groups. The positive control group showed increased fetal/litter incidence of spina bifida (12/4), cranioschisis (10/4) and protruding tongue (4/2). One runt and one fetus with no tail and no anal opening were also observed. TB-I notes that for several fetuses identified grossly with spina bifida, skeletal examinations did not indicate spina bifida.
2. Visceral Examination - No visceral abnormalities were observed among vehicle control or treatment groups. In the aspirin-treated positive control group, single incidences of microphthalmia and missing or undeveloped kidney were observed in separate litters.
3. Skeletal Examination - There were no significant treatment-related skeletal variations/malformations observed. A small number of fetuses exhibited delayed vertebral ossification at high dose and bifid vertebral centra at low dose, but were not considered treatment-related due to the small number of fetuses affected (4 in each case, in 3 litters) and the occurrence of these variations in isolation. Numerous skeletal abnormalities were observed in the aspirin-treated group.

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TABLE 4. Skeletal Examinations<sup>a</sup>

Observations <sup>+</sup>	Dose (mg/kg/day)				
	0	Aspirin/250	PNP/1.4	PNP/13.8	PNP/27.6
#Fetuses(litters) examined	90 (14)	61 (11)	94 (15)	95 (15)	68 (10)
OBSERVATION:	#Fetuses(litters) affected				
Vertebra					
delayed ossification	0	41(7)	0	1	4(3)
bifid vertebral centrum	0	0	4(3)	2(1)	1
scrambled centrum	0	7(3)	0	0	0
irregular arches	0	3(1)	0	0	0
Spina bifida	0	2(2)	0	0	0
Ribs					
14 pair	1	0	0	0	0
15 pair	0	1	0	0	0
unequal count	0	1	0	0	0
wavy	0	1	0	0	0
fused	0	3(3)	0	0	0
Cranial bones, unossified	0	10(3)	0	0	0
Sternum, scrambled	0	3(1)	0	0	0
Malformation index (%)	0	58.33*	0	0	0
Variation index (%)	6.67	66.67*	20.00*	12.5	35.35*

+ Some observations may be grouped together

a Data extracted from Appendix D of study report 75-51-0047-79-F (summarized from individual data by reviewer). Not analyzed statistically except for malformation/variation indices.

## III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: The study authors concluded that maternal toxicity was observed at the highest dose tested (27.6 mg/kg/day) based on decreased body weight/body weight gain and that no developmental toxicity was observed at any dose tested.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Maternal toxicity in the form of reduced mean body weight and body weight gain was observed at the highest dose tested, 27.8 mg/kg/day. Food consumption data were not provided. No clinical signs were reported. The maternal toxicity NOEL is 13.8 mg/kg/day and the LOEL is 27.8 mg/kg/day.
2. DEVELOPMENTAL TOXICITY: There were no apparent developmental effects observed with the possible exception of delayed ossification of the vertebrae at high dose; however, since no other ossification sites were affected, only 4 fetuses were affected and interpretation of the data was complicated by the

## Paranitrophenol

Developmental Study OPPTS 870.3700 [§83-3(a)]

reduced number of litters available, TB-I agreed with the study authors that this was not a treatment-related effect. The aspirin positive control group showed increases in numerous skeletal variations and also an increase in spina bifida and soft tissue abnormalities.

- a. Deaths/Resorptions: No apparent effect
- b. Altered Growth: No apparent effect
- c. Developmental Variations: No apparent effect. A statistically significant increase was reported for the variation index (percent litters affected by some type of variation) in the low and high dose groups. The increase at low dose was due to increased incidence of bifid vertebral centra, which was observed in 4 fetuses of 3 litters, did not show a dose response. At high dose, delayed ossification of vertebrae was observed in 4 fetuses in 3 litters. This effect was not considered treatment-related for the reasons outlined above.
- d. Malformations: No apparent effect

- C. STUDY DEFICIENCIES: There were numerous data and descriptions of procedures lacking in the study report and also experimental deficiencies, making interpretation of the study results difficult.
- insufficient numbers of litters, particularly at high dose
  - food consumption not measured
  - no indication as to whether animals were given careful daily examinations for clinical signs
  - dosing solutions not analyzed for stability, homogeneity or concentration
  - numerous data parameters were only given as individual animal data and not summarized
  - several parameters not analyzed statistically and methods used for statistical analysis not indicated
  - some numerical errors in the study tables, footnote for one of the methods used (footnote #3) missing.

Although this study is considered Supplementary, TB-I does not consider a new rat developmental toxicity study necessary at this time since there was no indication of any fetal toxicity at doses below the maternal toxicity LOEL of 27.6 mg/kg/day. The number of litters was lower than guideline recommendations, particularly at high dose (10), but 15 to 16 litters at the other dose levels should provide adequate numbers for evaluation and determination of a NOEL for developmental toxicity. Because of the above study deficiencies and the resulting uncertainty in interpretation of this study, the NOEL for developmental toxicity is  $\leq 26.5$  (LOEL not determined). The Registrant should submit information regarding dosing solution analysis for this study, if available, or information regarding the solubility of paranitrophenol in propylene glycol.

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TABLE II  
EVALUATION OF DATA

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The following indices were evaluated for each dosage group:

Index of fertility:  $\frac{\text{pregnant animals}}{\text{mated animals}} \times 100$

Index of viability:  $\frac{\text{alive fetuses}}{\text{total number of fetuses}} \times 100$

Index of death:  $\frac{\text{dead fetuses}}{\text{total number of fetuses}} \times 100$

Index of resorptions:  $\frac{\text{total resorptions}}{\text{total implantations}} \times 100$

Index of malformation:  $\frac{\text{litters w/ malformations}}{\text{total number of litters}} \times 100$

Index of variants:  $\frac{\text{litters w/ variation}}{\text{total number of litters}} \times 100$

Early resorption: implantation site only

Late resorption: fetal and/or placental remains

Runt: Any fetus weighing less than 70 percent of the average weight of its litter.



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**Chemical:** 4-Nitrophenol

**PC Code:** 056301  
**HED File Code** 13000 Tox Reviews  
**Memo Date:** 04/04/96  
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